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REMARKS

Claims 1-20 are pending in the instant application. The rejections set forth in the Office Action are traversed by argument below.

1. Rejection of claims 1 and 2 under 35 U.S.C. § 102

The Office Action asserts a rejection of claims 1-20 under 35 U.S.C. § 102(e), as being anticipated by U.S. Patent No. 6,433,145 (the '145 Patent). The Action accurately states that the '145 Patent discloses an amino acid sequence (*i.e.*, the amino acid sequence set forth in SEQ ID NO: 2 of the '145 Patent) that is identical to the amino acid sequence set forth of SEQ ID NO: 5 of the instant application. The Action also states that the '145 Patent discloses variants; pharmaceutically acceptable carriers; the fragment set forth in SEQ ID NO: 6 of the instant application; derivatives, including polymers; fusion proteins; and expression in eukaryotic and prokaryotic cells.

Applicants submit a Declaration under 37 C.F.R. § 1.131 establishing conception of the subject matter of the claims rejected under 35 U.S.C. § 102(e) prior to the effective date of the reference on which the rejection is based, as well as establishing that the subject matter of the rejected claims was diligently reduced to practice. Applicants note that due to the unavailability of some of the named inventors, Applicants' representative was unable to secure an executed Declaration. However, Applicants' representative will secure and promptly submit an executed Declaration containing the signatures of all three named inventors. Applicants contend that because the Declaration sufficiently establishes the conception of the subject matter of the claims prior to the effective date of the '145 Patent, and further, establishes that the subject matter of the rejected claims was diligently reduced to practice, the Declaration is sufficient to overcome the rejection of claims 1-20 under 35 U.S.C. § 102(e) as being anticipated by the '145 Patent. Applicants, therefore, respectfully request that this ground of rejection be withdrawn.

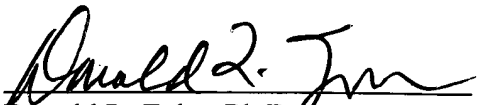
CONCLUSIONS

If Examiner Andres believes it to be helpful, she is invited to contact the undersigned representative by telephone at 312-913-0001.

McDonnell Boehnen Hulbert & Berghoff LLP
300 South Wacker Drive
Chicago, Illinois 60606
312-913-0001

Respectfully submitted,
McDonnell Boehnen Hulbert & Berghoff

Dated: June 10, 2004

By: 
Donald L. Zuhn, Ph.D.
Reg. No. 48,710

McDonnell Boehnen Hulbert & Berghoff LLP
300 South Wacker Drive
Chicago, Illinois 60606
312-913-0001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
(Case No. 99-372-F)

PATENT

In re Application of:	**Welcher et al.**	)
Serial No.:	**09/927,850**	) **Before the Examiner: J. Andres**
Filed:	**August 10, 2001**	) **Group Art Unit: 1646**
For:	**Interferon-Like Molecules and Uses Thereof**	)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION PURSUANT TO 37 C.F.R § 1.131

We, Andrew A. Welcher, residing at 1175 Church Street, Ventura, California; Duanzhi Wen, residing at 3885 Campus Drive, Thousand Oaks, California; and Michael Kelly, residing at 790 San Doval Place, Thousand Oaks, California; hereby declare:

1. We are named co-inventors on United States Application No. 09/927,850, filed on August 10, 2001.

2. The invention disclosed and claimed in the instant patent application was conceived in the United States by us before July 21, 1998 and was then diligently reduced to practice.

3. Accompanying this Declaration are photocopies of forty-one (41) pages from our laboratory notebook showing conception of our invention before July 21, 1998. Specifically, the photocopies of our laboratory notebook show that a genomic cloning approach was used to identify the nucleic acid sequence of human interferon-like polypeptide (*see* page 34 of laboratory notebook). Three genomic clones were identified as containing nucleic acid sequences encoding at least a portion of human interferon-like polypeptide (*i.e.*, clones 2, 6, and 7; *see* page 40). The nucleic acid sequences from these clones were isolated and then re-cloned into a suitable sequencing vector. One of the three genomic clones was determined to contain a partial nucleic acid sequence for human interferon-like polypeptide and another genomic clone (*i.e.*, clone 6) was determined to contain a full-length nucleic acid sequence for human interferon-like polypeptide (*see* page 62). The amino

acid sequence of human interferon-like polypeptide was determined from the latter nucleic acid sequence.

4. The dates on the laboratory notebook pages have been redacted from the photocopies. However, the dates are before July 21, 1998, the date on which U.S. Provisional Application No. 60/093,643 was filed, from which U.S. Application No. 09/487,792 claims the benefit of priority, from which U.S. Patent No. 6,433,145 issued on August 13, 2002.

5. Also accompanying this Declaration are photocopies of ten (10) pages from a Research Summary showing that the invention disclosed and claimed in the instant patent application was diligently reduced to practice. Specifically, the photocopies of the Research Summary show that experiments were performed in order to determine the function of protein encoded by the nucleic acid sequence described in paragraph 3 above, and that once the function of the protein had been determined, a Research Summary was prepared and submitted to the legal department of Amgen Inc., the assignee of the instant application. More particularly, photocopies of the Research Summary show that several versions of the human and rat IFN-L proteins were produced in a mammalian expression system (*see* page 7) and that rat IFN-L:Fc fusion protein treatment of several cell lines was found to cause phosphorylation of cellular proteins (*see* page 10).

6. The dates on the Research Summary pages have been redacted from the photocopies.

7. We hereby declare further that all statements made herein by each of us to our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: June 10, 2004

Signed: _____
Andrew A. Welcher

_____
Duanzhi Wen

_____
Michael Kelly

Page No.

[redacted] hrpe3-00078-F6.

① related to rIFNβ (30%).

② ⊕ in Certain Lot of pancreas mRNA (human) Northern. (J Cao).

③ Screening of pancreas cDNA (human) Library ⊖.

? Different Lot of RNA source

? Different level of expression

Decision: ① re-Screening human pancreas Library

② determine the genomic screening Condition

③ Is genomic screening feasible?

[redacted] IFN probe for genomic blot

17

PAGE: 1

[redacted] 13:16

ID: CHERENCOV 0.5
USER: 2 COMMENT:

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# :YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	655.1	427775.8	0.43	0.00	0.83

4.3×10^5 cpm/λ

To Page No.

d & Understood by me Date

Page No.___

[redacted]

PCR-Hot rIFN probe: template 1λ
1795-01 20pm
1795-02 1λ
10X PCR Buf. 10λ
10mM dNTPs 10λ
^{32}P-dCTP 5λ
25mM Mgcl$_2$ 16λ
Taq 1λ
H$_2$O to 100λ

94°C 30sec, 50°C 30sec 74°C 1min for 40 cycles.

G-50 column purified.

count: 4.3×10^5 cpm/λ.

Determine the hybridization and washing condition for genomic screening.

① very likely the homology of hIFN vs. rIFN is in the neighborhood of 60%.

② The formamide should be between 25% - 30%

. Washing condition should start with gentle wash, then elevate the T wash. Determine a condition in which fragment is detectable while background is minor.

To Page No.____

lessed & Understood by me,	Date	Invented by	Date

[redacted]

From the genomic southern (D. Wen keeps all the photo) it is clear:

① there is an 1.8kb HindIII fragment that strongly hybridize w/ probe.

② The formamide concentration can be adjusted to ~30% for relatively optimal signal vs. noice ratio.

③ Washing can be conducted @ ~55°C in 0.2 OR 0.3X SSC, 0.1% SDS.

Wen further found in literature:

① IFNλ one family member is single gene, ie. no intron.

② A lot of pseduo IFN gene as well.

But what about we identify a gene resemble pnrpe3-0078-F6, also proved its expressing tissues?

Page No.___

[redacted] Screening h Pancreas Library w/ Rat Probe

MIN - probe for human pancreas Library screening PAGE: 1

[redacted] 10:24

ID: CHERENCOV 0.5
USER: 2 COMMENT:

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# :YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	606.2	827666.2	0.31	0.01	0.91

PCR as on p. 35.
Library screening
- 1x10^6 clone on 20 plates.
- 30% formamide. 5xSSC. 42°C O/N
- Wash: 2x2xSSC, 0.1% SDS 30min @ 50°C
- O/N exposure

And

There is no double positive clones.

Issed & Understood by me,	Date	Invented by	Date

e No.__

[redacted] Screening human fibroblast genomic Library.

Library : human lung fibroblast genomic Library in FixII vector (Stratagene).

1×10^6 independent clones on 10 Nitro-cellulose membrane.

PCR probe: as described on p.35

-198
IFN probe for genomic Screening

PAGE: 1

[redacted] 12:47

ID: CHERENCOV 0.5
ER: 2 COMMENT:

ESET TIME :	0.50						
TA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
UNT BLANK :	NO	IC# :YES	REPLICATES :	1	RS232	: OFF
O PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
INTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
W LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

OTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

AM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	632.0	704931.5	0.34	0.01	0.89

Lift Library to Nitrocellulose membrane.

① 5 min denature, 5 min neutralization. 1 Sec 2X SSC

② 80°C bake 1 hr.

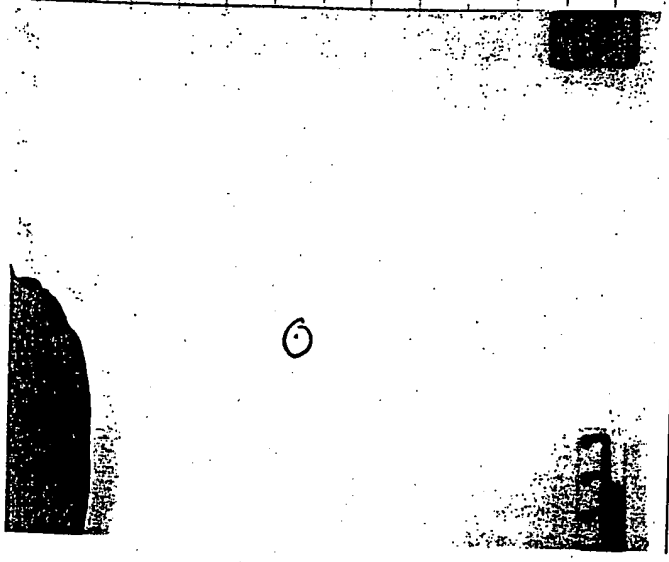
③ pre-hyb: 30% Formamamide
5X SSC
2X Denhart's
100 µg/ml ssDNA
0.1% SDS
2mM EDTA
0.1% Pyrophosphate

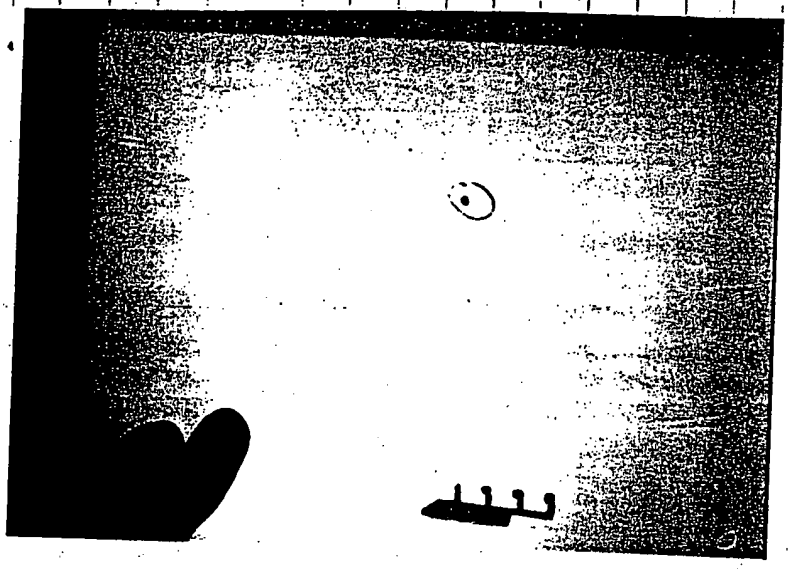
42°C O/N

④ Wash:
At R.T. 1X SSC, 0.1% SDS. 2X 30 min
At 55°C 0.2X SSC, 0.1% SDS 15 min
-80°C BioMax Kodak film O/N at -80°C

Page No.

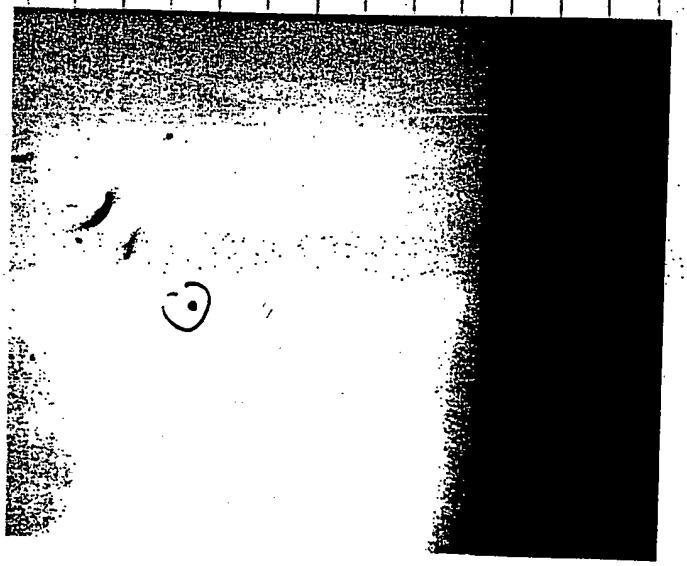
[redacted] Primary screening Result.





#2

#6



① pick up the ϕ colony with SM elution 37°C 2hrs.

② 1/500 dilution for secondary

③ double-lift secondary screening membrane.

#7. All double positive

Page No.

[redacted] 2nd screening > 6 IZN

4/19/98
IZN probe

PAGE: 1

ID: CHERENCOV 0.5 [redacted] 20:13
USER: 2 COMMENT:

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# : YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	615.7	458314.7	0.42	0.01	0.86

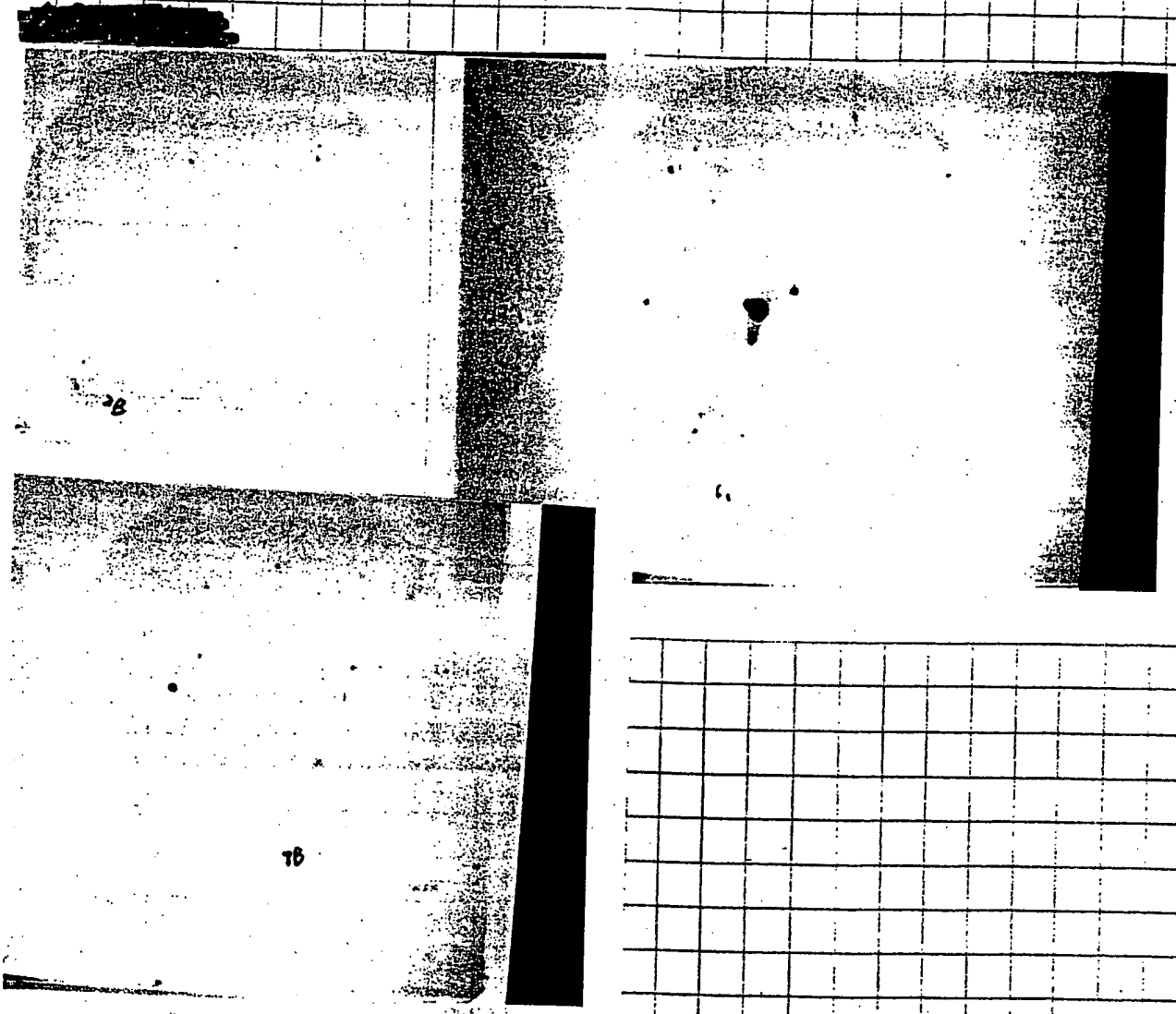
Hybridization: 20% formamide
5X SSC
42°C o/N

Wash: 1X SSC, 0.1% SDS R.T. 30min

0.2X SSC, 0.1% SDS 55°C 15min

To Page No.

essed & Understood by me,	Date	Invented by	Date



pick-up 2nd clone. elute with SM.
prepare for phage DNA preparation.

[redacted] Φ DNA preparation protocol;

aration of lamdan phage DNA
row up the phage (on plates or in liguid medim).
llecte the supernantat and spin down with 3ooorpn.
dd DNaseI (10u/ml) and RNase(20mg/ml)incubate 30 min at
c degree.
d 1/3 volume 30%PEG 6000 in3M NaCL put in ice for 1 hr or at 4
degree overnight.
10000g centrifugation 20 min
iscard supernatant and interverte the tube on papers to remove
G completely.
d 1/10-1/5 of origin volume T10E1 to suspend the phage
ticles.
d 0.5M EDTA to a final concentration of20mM and proteinaseK
mg/ml ,incubate 1hr in 55c degree waterbath.-
id 5%CATBin 0.5M NaCL to a final concentration of 0.1% and put
65c degree for 5 min.
sin down and dissolve in 1.2M NaCL .
Add 2.5vol.of enthanol and spin down.
liscard the enthanol and wash once with 70% enthanol.
.r dry the pellets and dissolve in T10E1 in ice 30 min. then at
ic degree for 10min. don,t put on RT. stock in 4c degree.

- phage has to be amplified once before proceeding to Φ DNA prep. on a 100mm plate. 10ul Φ + top agar → o/n 37°c.
SM elute
- on LB/Agarose plate, grow Φ o/n @ 37°C
- elute w/ SM 2 1/2 hrs @ 7-7.

nessed & Understood by me,	Date	Invented by	Date

e No.__ [redacted]

[redacted]

Human Genomic IFN like clone

BECKMAN DU-600

Φ prep:

Date: [redacted]
Time: 23:19

Leic Acid
adSamples Method SaveClear Print Quit

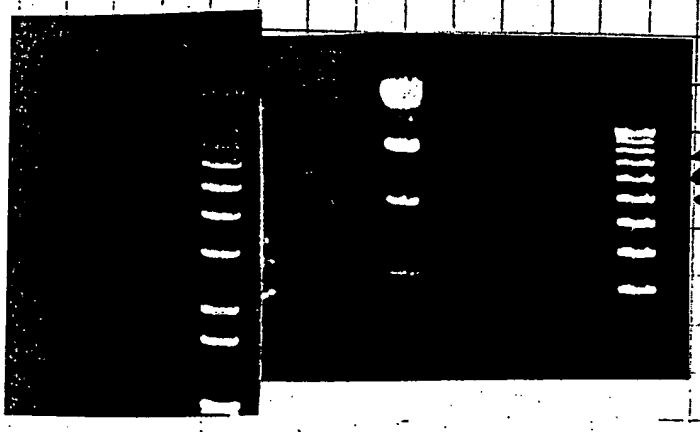
esults file: A:\WORK_RES

Method name: A:\DEFAULT

ssay type: General Ratio and Concentration
ormula setup: VIEW
ampling device: None
ead average time: 0.50 sec

Units: UG/UL
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [No]

ample	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	x100 ssRNA UG/UL	X100 dsDNA UG/UL
#2	0.1428	0.0774	1.8453	0.5419	0.5711	0.7139
#6	0.3206	0.1775	1.8066	0.5535	1.2823	1.6029
#7	0.1731	0.0913	1.8962	0.5274	0.6922	0.8653



#2: 143 ng/λ
#6: 32.1 ng/λ
#7: 17.3 ng/λ

Set-up NotI digestion to release insert in Fix II.

20 mg Φ DNA
10X H
Not I (HC) 40u x 2.5 = 100
H2O

ON @ 37°C incubator.

[redacted] where is the IFNt fragment?

The two ← indicated on p.49 (insert of interest)

Repeat Not I digestion, run a small agarose 0.5% gel.

Transfer and Southern to determine the band

Hyb: 30% formamide, wash: 0.2xSSC, 0.1% SDS.
55°C 15min

Results on p.46

BECKMAN DU-600

Date: [redacted]
Time: 16:18

ucleic Acid
ReadSamples Method SaveClear Print Quit

Results file: A:\WORK_RES

Method name: A:\DEFAULT

Assay type: General Ratio and Concentration
Formula setup: VIEW
Sampling device: None
Read average time: 0.50 sec

Units: UG/UL
Background Correction: [No]
Concentration: [Yes]
Peak Pick: [No]

1/20

Sample ID		abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	x100 ssRNA UG/UL	X100 dsDNA UG/UL
1	#2	0.0059	0.0027	2.1876	0.4571	0.0235	0.0293
2	#6	0.0082	0.0043	1.8895	0.5292	0.0326	0.0408
3	#7	0.0085	0.0046	1.8522	0.5399	0.0338	0.0423
4							

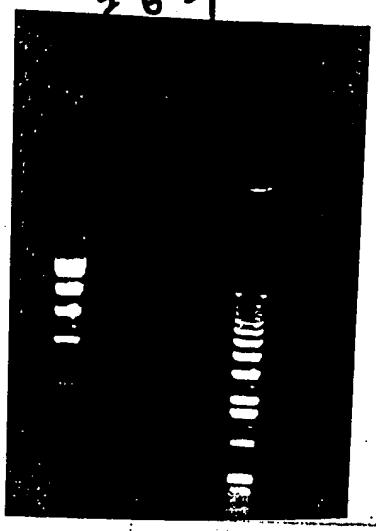
#2 5.9 x 50 x 20 = 6ng/λ
#6 8.2 x 1000 = 8ng/λ
#7 8.5 x 1000 = 8.5ng/λ

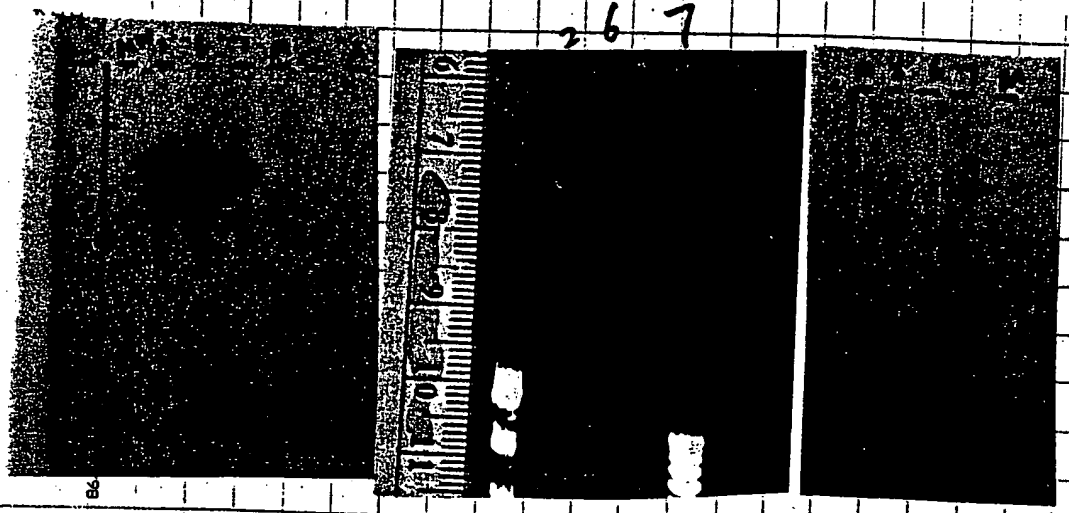
Ligation: 10ng cut vect.
+ 6λ Insert
+ 2λ 10x Buff
5u T4 ligase

essed & Understood by me, | Date | Invented by | Date

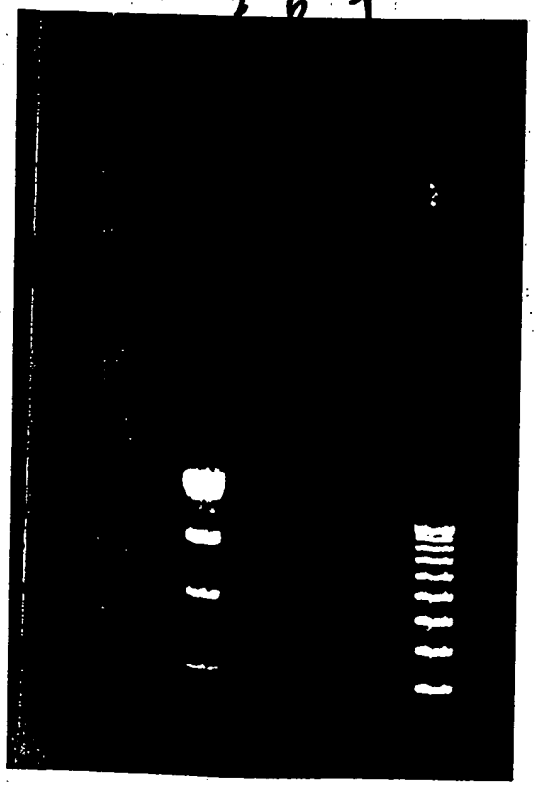
Page No.

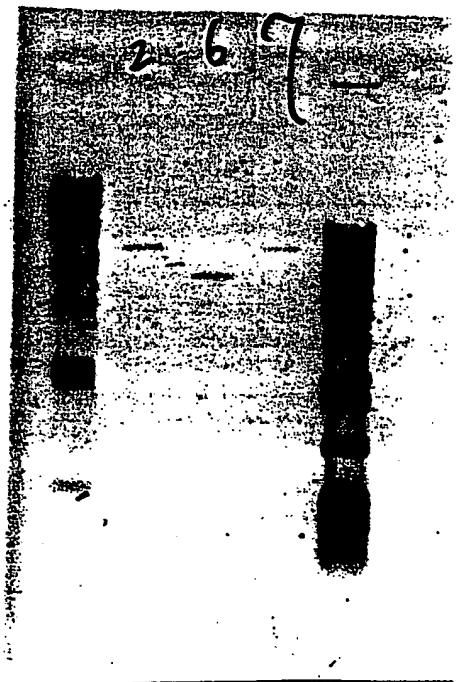
[redacted]





[redacted] Gel purify IFN genomic fragment





d & Understood by me.	Date	Invented by	Date

Page No.__

pSV.Sport/IFN genomic fragment

BECKMAN DU-600

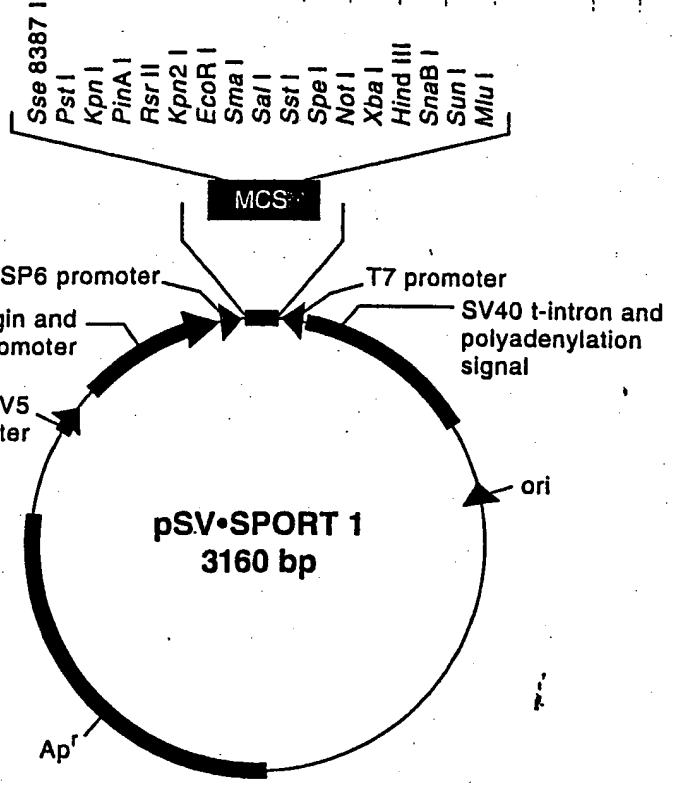
cleic Acid
eadSamples Method

Results file: A:\WORK_RES

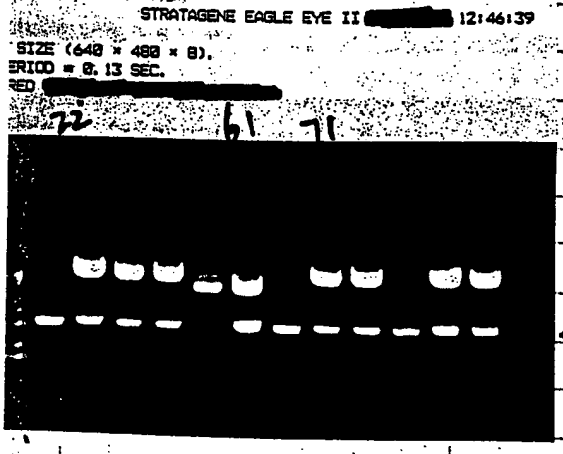
Assay type: General Ratio and Concen
Formula setup: VIEW
Sampling device: None
Read average time: 0.50 sec

Sample ID	abs 260.0 nm	abs 280.0 nm
1 psv.psport HindIII	0.0107	0.0039
2	0.0204	0.0093
3 psv.psport NotI		

psv.psport/NotI 20 ng/λ



0.8% TAE-agarose gel/NotI digestion



miniprep DNA 5μl
10X H 1 μl
NotI 0.5μl (10u)
H2O 3.5μl
Total 10 μl @ 37°C 1½ hrs.

ssed & Understood by me,	Date	Invented by	Date

ge No.__

[redacted] Set-up genomic clone Analysis.

5λ mini-prep DNA
1λ 10x Buffer (BMB)
0.5λ EZ.
3.5λ H2O
10λ @ 37°C 1 1/2 hrs.

8% TAE gel.

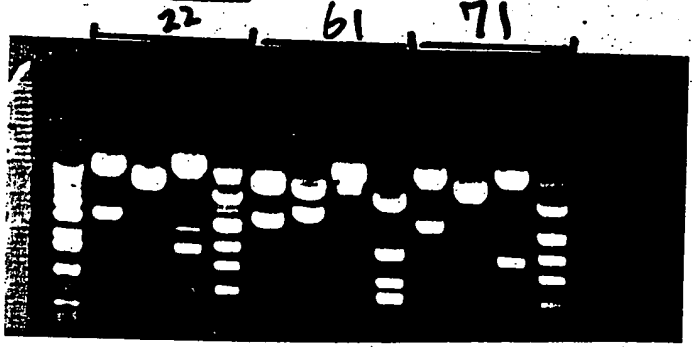
Restriction EZs:
present on MCS of psv.pspart
NotI, EcoRI, XbaI & HindIII

22 and 71 are identical independent clones. but not 61.

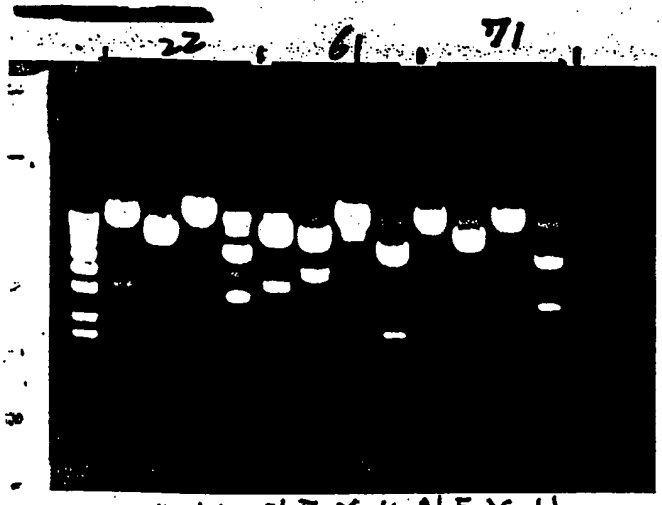
	22	61	71
EcoRI	0	2	0
XbaI	3	2	3
HindIII	>4	>4	>4

Is that why initially EcoRI digestion on genomic DNA didn't light up by rmrep3 probe?

STRATAGENE EAGLE EYE II [redacted] 15:10:42
IE (640 × 480 × 8).
ID = 0.46 SEC.

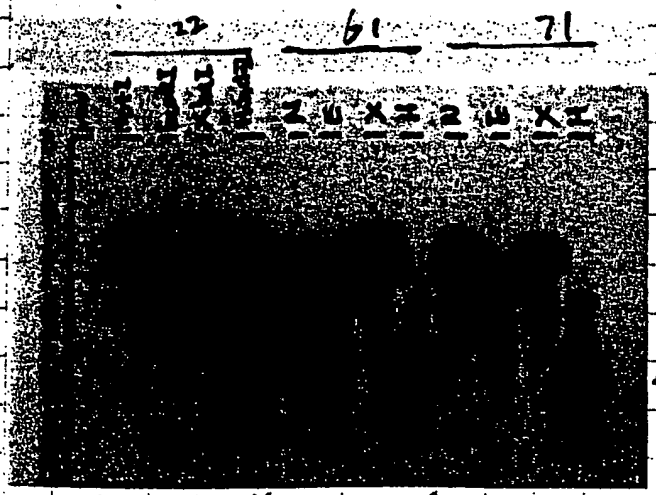


STRATAGENE EAGLE EYE II [redacted] 15:35:05
540 × 480 × 8).
0.66 SEC.



MW EcoRI XbaI HindIII NEXHNEXH

Transfer this gel for alkaline transfer.



* HindIII incomplete digestion

Page No.

[redacted] Washing Southern Blot.

Hybridization: 30% Formamide, 5x SSC, 2x Denhart's
100µg SSDNA, 0.2% SDS, 2mM EDTA, 0.1% pyrophos
(No NaH_2PO_4)
42°C, 3 hrs. + 5% Dextran Sulfate for Hyb.

PCR-Hot probe: Template 1λ (20ng)
1795-01 1λ (20pm)
1795-02 1λ (20pm)
10mM dNTP 10λ (dCTP@ 0.1mM)
^{32}P-dCTP 5λ
10x PCR Buf. 10λ
25mM $MgCl_2$ 16λ (4mM final)
Taq 1λ
H_2O to 100µl Control: +60µl H_2O
with 10mM dNTPs

5/7/98 rIFN probe

PAGE: 1

ID: CHERENCOV 0.5
USER: 2 COMMENT:
[redacted] 22:04

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# : YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:		none		

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	592.9	528289.8	0.39	0.01	0.86

1ml Count.

Washing: 1 x SSC. 0.1% SDS @ RT 1hr
0.2 x SSC, 0.1% SDS @ 55°C 15min
exp. @ -80°C. O/N.

ssed & Understood by me,	Date	Invented by	Date

le No.___

[redacted] h mrep-3 cloning: Subclone genomic fragment.

Set-up digestion:

70λ mini-prep DNA
8λ 10x B Buffer (BMB)
2λ BamHI (20u)
80λ total @ 37°c 5 hrs

8λ run gel (with dye)

◀ indicates positive band in Southern Blot with mrep-3 probe (p. 492.

92λ (with dye) run a 8% TAE gel.

Qiagen Gel Purification Kit

No. 61 No. 71

0.8% TAE-agarose gel (HindIII fragments)

BECKMAN DU-600

Date: [redacted]
Time: 21:25

leic Acid
adSamples Method SaveClear Print Quit

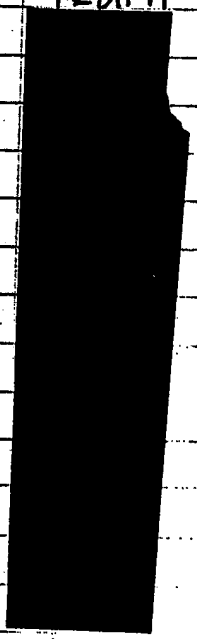
esults file: A:\WORK_RES Method name: A:\DEFAULT

ssay type: General Ratio and Concentration Units: UG/UL
ormula setup: VIEW Background Correction: [No]
ampling device: None Concentration: [Yes]
ead average time: 0.50 sec Peak Pick: [No]

1/10 dilu

ample	abs 260.0 nm	abs 280.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	x100 ssRNA UG/UL	X100 dsDNA UG/UL
#61	0.0168	0.0093	1.7933	0.5576	0.0670	0.0838
#71	0.0201	0.0112	1.7963	0.5567	0.0803	0.1003

No. 61.71



61.71 insert is probably same (identical size, HindIII fragment size)

#61 0.0168 × 50 × 20 ≈ 17ng/λ

#71 0.0206 × 50 × 20 ≈ 20ng/λ

& Understood by me. Date Invented by

[redacted] Ligation:

3λ dephosphorylated psv-psport (17ng)
1λ 10x Lig. Butter (BMB)
1λ T4 DNA Ligase (BMB)
5λ Insert (85 ~ 100ng)
10λ

Control: +5Nl H2O in vector control.

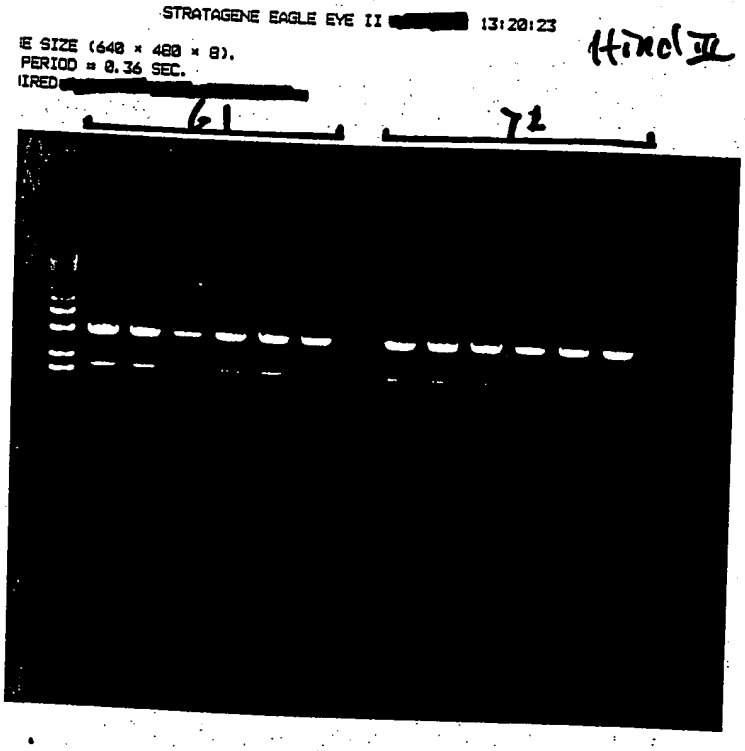
14°C 1hr

Transformation: 40λ DH10α ElectroMax cell
+ 1.5λ Ligation Mix

6 transformation clones/each for mini-prep.

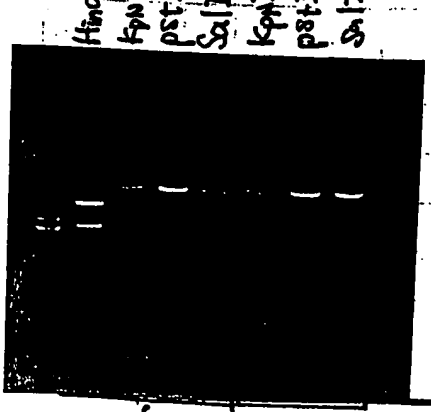
Digest w HindIII
5λ DNA
1λ 10XB (BMB)
0.5λ HindIII (5u)
3.5λ H2O
10λ total 37°C 1½hrs

0.8% TAE gel



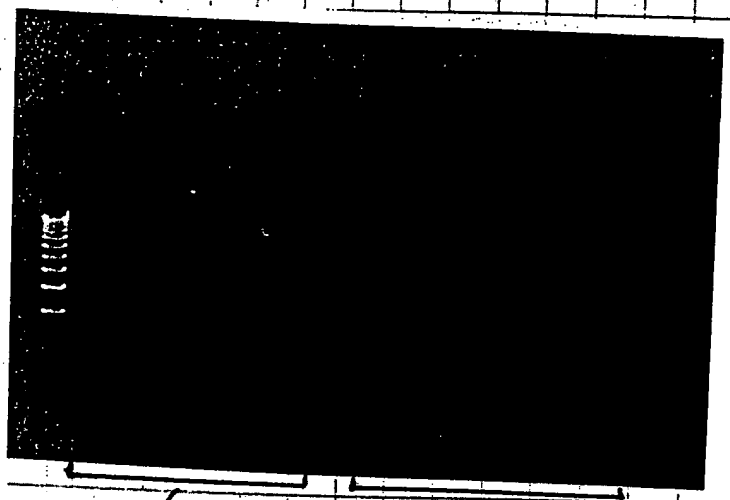
All contain insert. (1.8Kb)

Further Analysis. w
KpNI. pstI. SalI (BMB)
5λ DNA
1λ 10x Buffer
0.5λ EZ (5u)
3.5λ H2O
10λ 37°C ½hr



So no additional EZ cut in the insert.

Witnessed & Understood by me, | Date | Invented by | Date



Transfer to N.C. for future reference.

61 71

Sequencing Request

ID	Clone	Requestor	Status	Submit	Rcvd
9808902	hgmrep3-6.1	Wen Chen	Pending	[redacted]	00/00/00
9808903	hgmrep3-6.2	Wen Chen	Pending	[redacted]	00/00/00
9808904	hgmrep3-6.5	Wen Chen	Pending	[redacted]	00/00/00
9808905	hgmrep3-7.1	Wen Chen	Pending	[redacted]	00/00/00
9808906	hgmrep3-7.2	Wen Chen	Pending	[redacted]	00/00/00
9808907	hgmrep3-7.2 X deleted!	Wen Chen	Pending	[redacted]	00/00/00
9808908	hgmrep3-7.3	Wen Chen	Pending	[redacted]	00/00/00

3-6.1, 3-6.5, 3-7.1 } identical Insert in same orientation

3-6.2, 3-7.2 } identical Insert in reverse orientation.

Page No.___

[redacted] mCmp2 Southern: Human Genomic DNA

probe: mCmp2 template 2λ (long)
1582-15 (5' primer) 2λ (20pm)
1536-79C (3' primer) 2λ (20pm)
10x Buffer 10λ
(10mM dNTP; dCTP@0.1mM) 10λ
α-^{32}PdCTP 5λ
25mM $MgCl_2$ 16λ
Taq (BMB) 1λ
H_2O 53λ
100λ

Cold control + 10λ 10mM dNTP + 5λ extra H_2O
94°C 30sec → 60°C 30sec → 72°C 1min → 45 cycles

PAGE: 1

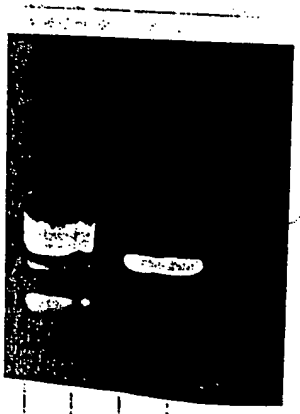
ID: CHERENCOV 0.5
USER: 2 COMMENT:
[redacted] 16:06

PRESET TIME :	0.50						
DATA CALC :	CPM	H# : NO	SAMPLE REPEATS:	1	PRINTER	: STD
COUNT BLANK :	NO	IC# :YES	REPLICATES :	1	RS232	: OFF
TWO PHASE :	NO	AQC : NO	CYCLE REPEATS :	1		
SCINTILLATOR:	XTAL	LUMEX: NO	LOW SAMPLE REJ:	0		
LOW LEVEL :	NO	HALF LIFE CORRECTION DATE:			none	

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	32P %ERROR	LUMEX %	ELAPSED TIME
1	**-1	0.50	602.8	1115564	0.27	0.02	0.95

1λ 1.1 x 10^6 cpm/λ



cold control.

G/ primary Screening:

To Page No. 60

Project No._____

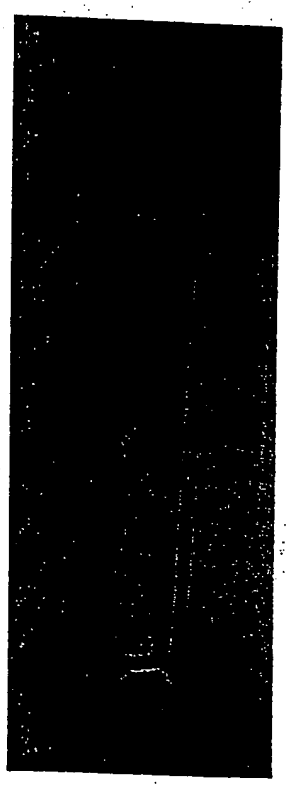
Book No._____ TITLE CMJ2 54

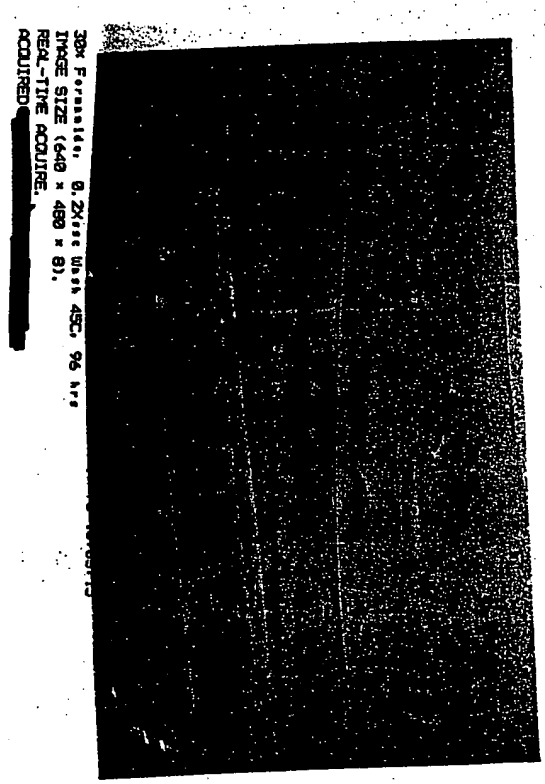
e No.__

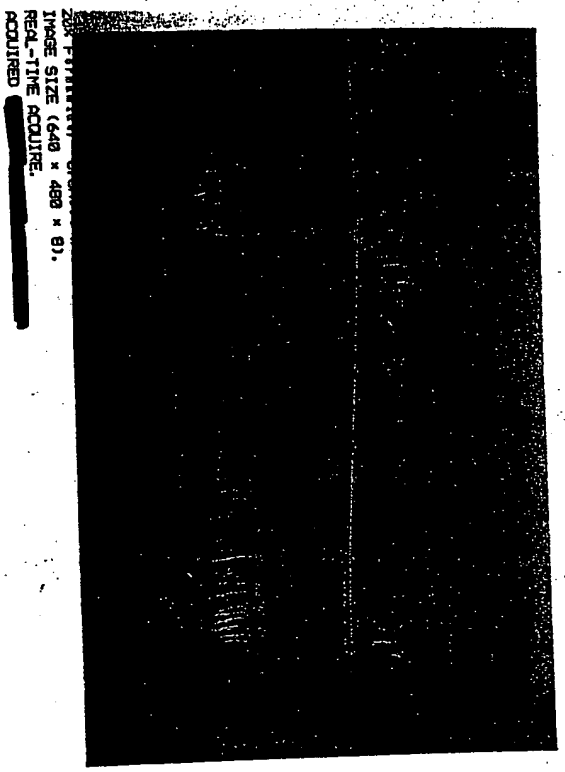
Hybridization in 30% Formamide

	Final	Stock
30% Formamide		~~30~~ 5 ml
5x SSC		5 ml
2x Denhardt's		4 ml
10 MG/ml SS DNA		1 ml
0.2% SDS		1 ml
2mM EDTA		0.4 ml
0.1% Pyrophosphate		1 ml
H2O to 100 ml		37.6 ml

42°C O/N . strips rehyb.







	30%	20%	20%
ection			
°C (Formamide)			
: SSC	0.2x	0.5x	0.5x
SDS	0.1%	0.1%	0.1%
°C exp	24 hrs	24 hrs	24 hrs

To Page No. 84

REVERSE-COMPLEMENT of: 9808902.Con check: 3852 from: 1 to: 1163
WChen
DNA=hgmrep3-6.1 p=890-24 end
assembled by JK: [redacted]
9808902.con

With 1 enzymes: HINDIII

[redacted] 17:01 ..

```
      CTGAGAAGAGTCACCTGGCAAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCT
    1 ---------+---------+---------+---------+---------+---------+ 60
      GACTCTTCTCAGTGGACCGTTTTAGACTCTGTAGACTCATCATACTCGTTAAGTAAAGGA

a       L  R  R  V  T  W  Q  N  L  R  H  L  S  S  M  S  N  S  F  P  -

      GTAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCCAAGAGTTTCTGCAATACACC
   61 ---------+---------+---------+---------+---------+---------+ 120
      CATCTTACAGATGCTCTTTTGTATCGAAAACTCAACGGGGTTCTCAAAGACGTTATGTGG

a       V  E  C  L  R  E  N  I  A  F  E  L  P  Q  E  F  L  Q  Y  T  -

      CAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAATGTCCCTACAGGCCTTCAAC
  121 ---------+---------+---------+---------+---------+---------+ 180
      GTTGGATACTTCTCCCTGTAGTTCTTCCGGAAGATACTTTACAGGGATGTCCGGAAGTTG

a       Q  P  M  K  R  D  I  K  K  A  F  Y  E  M  S  L  Q  A  F  N  -

      ATCTTCAGCCAACACACCTTCAAATATTGGAAAGAGAGACACCTCAAACAAATCCAAATA
  181 ---------+---------+---------+---------+---------+---------+ 240
      TAGAAGTCGGTTGTGTGGAAGTTTATAACCTTTCTCTCTGTGGAGTTTGTTTAGGTTTAT

a       I  F  S  Q  H  T  F  K  Y  W  K  E  R  H  L  K  Q  I  Q  I  -

      GGACTTGATCAGCAAGCAGAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAAT
  241 ---------+---------+---------+---------+---------+---------+ 300
      CCTGAACTAGTCGTTCGTCTCATGGACTTGGTTACGAACCTCCTTCTGCTCTTACTTTTA

a       G  L  D  Q  Q  A  E  Y  L  N  Q  C  L  E  E  D  E  N  E  N  -

      GAAGACATGAAAGAAATGAAAGAGAATGAGATGAAACCCTCAGAAGCCAGGGTCCCCCAG
  301 ---------+---------+---------+---------+---------+---------+ 360
      CTTCTGTACTTTCTTTACTTTCTCTTACTCTACTTTGGGAGTCTTCGGTCCCAGGGGGTC

a       E  D  M  K  E  M  K  E  N  E  M  K  P  S  E  A  R  V  P  Q  -

      CTGAGCAGCCTGGAACTGAGGAGATATTTCCACAGGATAGACAATTTCCTGAAAGAAAAG
  361 ---------+---------+---------+---------+---------+---------+ 420
      GACTCGTCGGACCTTGACTCCTCTATAAAGGTGTCCTATCTGTTAAAGGACTTTCTTTTC

a       L  S  S  L  E  L  R  R  Y  F  H  R  I  D  N  F  L  K  E  K  -

      AAATACAGTGACTGTGCCTGGGAGATTGTCCGAGTGGAAATCAGAAGATGTTTGTATTAC
  421 ---------+---------+---------+---------+---------+---------+ 480
```

```
    TTTATGTCACTGACACGGACCCTCTAACAGGCTCACCTTTAGTCTTCTACAAACATAATG
    K  Y  S  D  C  A  W  E  I  V  R  V  E  I  R  R  C  L  Y  Y  -
    TTTTACAAATTTACAGCTCTATTCAGGAGGAAATAAGGTATATTTTTGGAATTAAAATTC
481 ---------+---------+---------+---------+---------+---------+ 540
    AAAATGTTTAAATGTCGAGATAAGTCCTCCTTTATTCCATATAAAAACCTTAATTTTAAG
    F  Y  K  F  T  A  L  F  R  R  K  *  G  I  F  L  E  L  K  F  -
    CTTTTCCCTCCGAAATCTCTTTCTCCTTCTCCTCCTCCATCTTCTTTTTAAGGATTGTTG
541 ---------+---------+---------+---------+---------+---------+ 600
    GAAAAGGGAGGCTTTAGAGAAAGAGGAAGAGGAGGAGGTAGAAGAAAAATTCCTAACAAC
    L  F  P  P  K  S  L  S  P  S  P  P  P  S  S  F  *  G  L  L  -
    TGCTGTCCTGTAAGCCTGTCCTCAGTTGGACTGGTAGCCTCGGAACATCAGGGACACTCA
601 ---------+---------+---------+---------+---------+---------+ 660
    ACGACAGGACATTCGGACAGGAGTCAACCTGACCATCGGAGCCTTGTAGTCCCTGTGAGT
    C  C  P  V  S  L  S  S  V  G  L  V  A  S  E  H  Q  G  H  S  -
    CCTCTCTAAGGAGAGGTAATGCCAACCATCCTCAGGGTGACCAAGAGTCTCCTTAGAAAG
661 ---------+---------+---------+---------+---------+---------+ 720
    GGAGAGATTCCTCTCCATTACGGTTGGTAGGAGTCCCACTGGTTCTCAGAGGAATCTTTC
    P  L  *  G  E  V  M  P  T  I  L  R  V  T  K  S  L  L  R  K  -
    TCTTTAAGACATTTTTAAAGGAATAAGATTCCCTCTCCGTCTTCTTCTATTCTCTCTTGC
721 ---------+---------+---------+---------+---------+---------+ 780
    AGAAATTCTGTAAAAATTTCCTTATTCTAAGGGAGAGGCAGAAGAAGATAAGAGAGAACG
    S  L  R  H  F  *  R  N  K  I  P  S  P  S  S  S  I  L  S  C  -
    TCTTTTCTGTGGCCATTTTGAAAGAGCTTTGCTATATATACCACCTGTGGACTTCACCAA
781 ---------+---------+---------+---------+---------+---------+ 840
    AGAAAAGACACCGGTAAAACTTTCTCGAAACGATATATATGGTGGACACCTGAAGTGGTT
    S  F  L  W  P  F  *  K  S  F  A  I  Y  T  T  C  G  L  H  Q  -
    GACAATGGCTAGAGGATAGGGAGCAGAGAATGTTGCAAAATGGTAACATTTCAATGACTT
841 ---------+---------+---------+---------+---------+---------+ 900
    CTGTTACCGATCTCCTATCCCTCGTCTCTTACAACGTTTTACCATTGTAAAGTTACTGAA
    D  N  G  *  R  I  G  S  R  E  C  C  K  M  V  T  F  Q  *  L  -
    AACTGTTTTGCTGCCAAGGTTGCTTATCCTATGAAAATTCAGCACATTAAAAGAGCTTAT
901 ---------+---------+---------+---------+---------+---------+ 960
    TTGACAAAACGACGGTTCCAACGAATAGGATACTTTTAAGTCGTGTAATTTTCTCGAATA
    N  C  F  A  A  K  V  A  Y  P  M  K  I  Q  H  I  K  R  A  Y  -
    ACATGCTCCCTAGAGTCAATACTCTTGCATTTTCCCCCTCCTGCTCGGGGGGAAAAAGGT
961 ---------+---------+---------+---------+---------+---------+ 102
```

To Page No._____

```
a       T  C  S  L  E  S  I  L  L  H  F  P  P  P  A  R  G  E  K  G  -

        TGACATTTCTGGCCCATTTCCTTCTCAGCTTGGTTTGTTTGAATTGATGCTTGTGGAATG
1021    ---------+---------+---------+---------+---------+---------+ 1080
        ACTGTAAAGACCGGGTAAAGGAAGAGTCGAACCAAACAAACTTAACTACGAACACCTTAC

INTRON>
INTRON>
a       *  H  F  W  P  I  S  F  S  A  W  F  V  *  I  D  A  C  G  M  -

        GTATTTCATTACTTTAAGAGTGAAGATCCATAGTGAAATTGGATGGATGGTTGAATTAGA
1081    ---------+---------+---------+---------+---------+---------+ 1140
        CATAAAGTAATGAAATTCTCACTTCTAGGTATCACTTTAACCTACCTACCAACTTAATCT

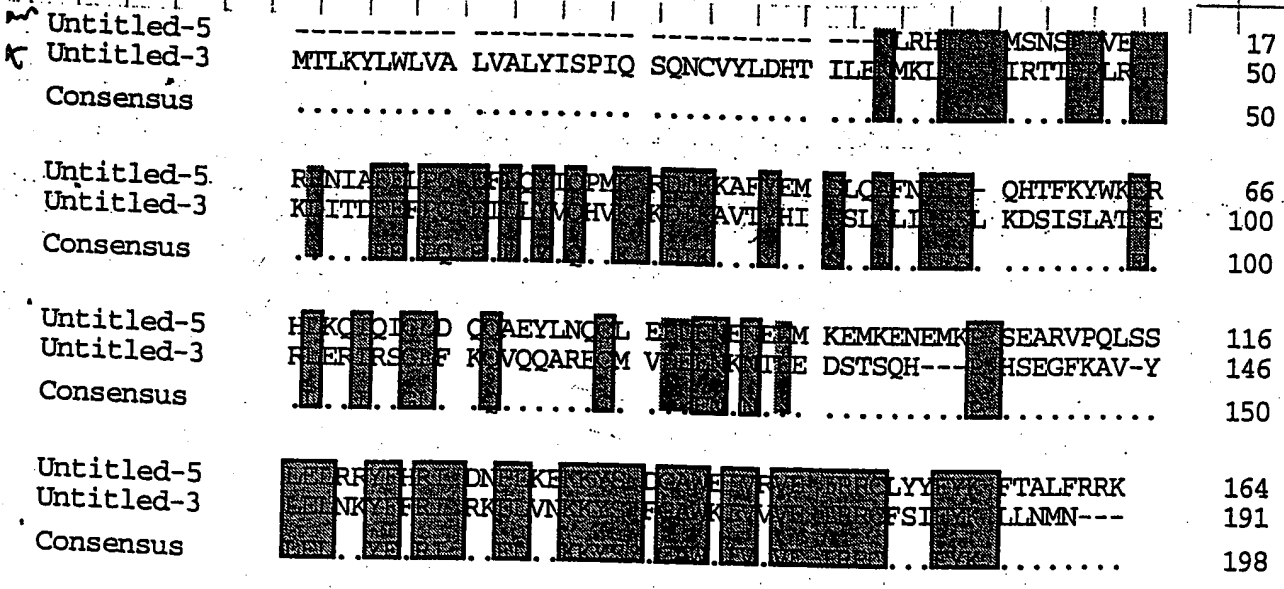
a       V  F  H  Y  F  K  S  E  D  P  *  *  N  W  M  D  G  *  I  R  -

          HindIII
             |
        CGACCATTAAGCTTACGTACGCG
1141    ---------+---------+--- 1163
        GCTGGTAATTCGAATGCATGCGC

a       R  P  L  S  L  R  T  -
```

Enzymes that do cut:

HindIII



essed & Understood by me,	Date	Invented by	Date

Page No.

Iomega	MALLFPLLAA LVMTSYSPVG SLGCDLPQNH GLLSRNTLVL LHQMRRISPF	50
I clone	---------- ---------- -----LRR-- --VTWQNLRH LSSMSNSFPV	21
ensus	....	50
Iomega	LCLKDRRDFR FPQEMVKGSQ LQKAHVMSVL HEMLQQIFSL FHTERSSAAW	100
I clone	EPLRENIAFE LPQEFLQYTQ PMKRDIKKAF YEMSLQAFNI F-SQHTFKYW	70
ensus	....	100
omega	NMTLLDQLHT GLHQQLQHLE TCLLQVVGEG E--------- ---SAGAISS	138
clone	KERHLKQIQI GLDQQAEYLN QCLEEDENEN EDMKEMKENE MKPSEARVPQ	120
ensus	....	150
omega	-PAILRRYFQ GIRVYLKEKK YSDCAWEVVR MEIMKSLFL STNMQERLRS	187
clone	LSSLFLRRYF HRLDNFLKEK KYSDCAWEIV RVEIRRCLYY FYKFTALFRR	170
ensus	....	200
omega	KDRDLGSS	195
clone	--------	171
ensus	....	208

IFN	MTLKYLWLVA LVALYISPIQ SQNCVYLDH- --TILENMKL LSSIRTTFRL	47
IFNomega	MALLFPLLAA LVMTSYSPVG SLGCDLPQNH GLLSRNTLVL LHQMRRISPF	50
IFN clone	---------- ---------- -----LRR-- --VTWQNLRH LSSMSNSFPV	21
nsensus	M.L....L.A LV....SP.. S..C.L...- --....N..L LSSMR..FP.	50
IFN	FPLKDITDRE FPQEILLYVQ HVKKDIKAVT YHISSLALII FSLKDSISLA	97
IFNomega	LCLKDRRDFR FPQEMVKGSQ LQKAHVMSVL HEMLQQIFSL FHTERSSAAW	100
IFN clone	EPLRENIAFE LPQEFLQYTQ PMKRDIKKAF YEMSLQAFNI F-SQHTFKYW	70
nsensus	...K..D.E FPQ..L.Y.Q ..K..DIK.V. YEMS.QAF.I F....S...W	100
IFN	TEERLERIRS GLFKGVQQAR ECMVEEENKN TEEDSTSQH- --PHSEGFKA	144
IFNomega	NMTLLDQLHT GLHQQLQHLE TCLLQVVGEG E--------- ---SAGAISS	138
IFN clone	KERHLKQIQI GLDQQAEYLN QCLEEDENEN EDMKEMKENE MKPSEARVPQ	120
nsensus	.E..L.QI.. GL.QQ.Q.L. .C..E.ENEN E........- --PS......	150
IFN	-VYLLNKYFR FRRKFLVRKE KYSDCAWEIV RVEMRRCFSI FYKLLNMN--	191
IFNomega	-PAILRRYFQ GIRVYLKEKK YSDCAWEVVR MEIMKSLFL STNMQERLRS	187
IFN clone	LSSLFLRRYF HRLDNFLKEK KYSDCAWEIV RVEIRRCLYY FYKFTALFRR	170
nsensus	-..L.LRRYF .R.R.FLKEK KYSDCAWE.V RVE.RRCL.. FYK.....R.	200
IFN	--------	191
Nomega	KDRDLGSS	195
N. clone	K-------	171
nsensus	K-------	208

Ratio: 2.484 Gaps: 4
Percent Similarity: 63.383 Percent Identity: 63.383

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

9808902.Con x Mrpe3-00078-F6-Wz.Ctg [redacted] 13:52 ..

```
1152 CACCTGGCAAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCTG 1103
     || || | ||||  ||| || |||||| || || || |   | |||||
 159 CATCTTGGAAAACATGAAACTTCTGAGCAGCATCAGGACCACCTTTCCCT 208

1102 TAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCCAAGAGTTTCTG 1053
     ||  ||||||| ||| | ||  | ||||||||| || |||||| |||||
 209 TAAGATGTCTAAAAGATATCACGGATTTTGAGTTTCCTCAAGAGATTCTG 258

1052 CAATACACCCAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAAT 1003
     |  |||  ||| | | |||| | |||||| |||   | |  |||| | ||
 259 CTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACCTATCATAT 308

1002 GTCCCTACAGGCCTTCAACATCTTCAGCC...AACACACCTTCAAATATT 956
      ||    | ||  | |  || ||||| |   || || || ||     .
 309 ATCTTCTCTGGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGG 358

 955 GGAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCA 906
      || ||||  || | |  |||  |||  |  ||||||    | ||||
 359 CGACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTCAAACAAGTG 408

 905 GAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACAT 856
      || |        | ||| |||  || || |||||  | |      |||
 409 CAGCAAGCTCGAGAGTGCATGGTAGACGAGGAGAACAAGA......ACAC 452

 855 GAAAGAAATGAAAGAGAATGAGATGAAACCCTCAGAAGCCAG.GGTCCCC 807
      | | |      ||   |  | || | | | ||   |  ||| || |  |
 453 GGAGG.......AGGACAGTACATCACAACATCCTCACTCAGAGGGCTTC 495

 806 CAGCTGAGCAGCCTGGAACTGAGGAGATATTTCCACAGGATAGACAATTT 757
      ||     |  ||||||| |||  |  ||||||  ||| ||    || ||
 496 AAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGAAAGTT 545

 756 CCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGTCCGAGTGG 707
      ||||  | | ||||||||||||  |||||||||| ||||||||   ||||
 546 CCTGGTAAATAAGAAATACAGTTTCTGTGCCTGGAAGATTGTCGTGGTGG 595

 706 AAATCAGAAGATGTTTGTATTACTTTTACAAATT 673
      |||| ||||||||||   |   |||||||| |
 596 AAATAAGAAGATGTTTCAGTATATTTTACAAACT 629
```

To Page No.

nessed & Understood by me,	Date	Invented by	Date

lo.__

Gaps: 4
Quality: 1170
lity Ratio: 2.484
Similarity: 63.383
Length: 484

N> type 9808902.pair
FIT of reverse of: 9808902.Con check: 3852 from: 1 to: 1163

gmrep3-6.1 p=890-24 end
bled by JK: [redacted]
02.con

Mrpe3-00078-F6-Wz.Ctg check: 4485 from: 1 to: 963

INTRON::DONGYINY [redacted] 16:07:43.44
WCHEN

INTRON::JCAO [redacted] 12:24:38.19
DONGYINY . . .

)l comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Swgapdna.Cmp
heck: 2335

Gap Weight: 50 Average Match: 10.000
Length Weight: 3 Average Mismatch: -9.000

Quality: 1170 Length: 484

Programme	Interferon Like Protein			
Investigator:	Duanzhi Wen			
Animal/Quanti	3 rabbits			
Immunogen:	Interferon like Protein			
Protocol:	MSU Standard			
Prog start date:				
Prog end date:	Ongoing			
Comments:	Give all sera to Duanzhi.			
ANIMAL# PI	[redacted]	50% Titer	[redacted]	50% Titer
#3429 5mls	30mls		30mls	
3432 5mls	30mls		30mls	
#3433 5mls	30mls		30mls	

Programme	Interferon Like Protein			
Investigator:	Duanzhi Wen			
Animal/Quanti	3 rabbits			
Immunogen:	Interferon like Protein			
Protocol:	MSU Standard			
Prog start date:				
Prog end date:	Ongoing			
Comments:	Give all sera to Duanzhi.			
NIMAL# PI	[redacted]	50% Titer	[redacted]	50% Titer
3429 5mls	30mls	1 1500	30mls	1 2000
432 5mls	30mls	1 2500	30mls	1 3000
3433 5mls	30mls	1 5000	30mls	1 10,000
[redacted]	50% Titer			
25mls	> 1 10,000			
25mls	> 1 10,000			
25mls	> 1 10,000			

essed & Understood by me, | Date | Invented by | Date

62

REVERSE-COMPLEMENT of: 9808903.Con check: 57 from: 1 to: 1894
WChen
DNA=hgmrep3-6.2
assembled by JK: [redacted]
9808903.con

1.8 kb Human IFN-like clone seq.

With 1 enzymes: HINDIII

[redacted] 15:37 ..

HindIII

```
      CGCGTACGTAAGCTTAATTTAACAAAATTGGAAAAACCTAAACTATACTGTGCTCTGGTG
    1 ---------+---------+---------+---------+---------+---------+ 60
      GCGCATGCATTCGAATTAAATTGTTTTAACCTTTTTGGATTTGATATGACACGAGACCAC

      ACCTAGCAATCAAATAATCACAGTCATTTGGTCAATGTCTATGATTAACTCAATGAGACA
   61 ---------+---------+---------+---------+---------+---------+ 120
      TGGATCGTTAGTTTATTAGTGTCAGTAAACCAGTTACAGATACTAATTGAGTTACTCTGT

      GGATGTTTGGCTATAGCACCAGGTACAAAAAATATATTTTCATGAAGGATCACTCCCTCT
  121 ---------+---------+---------+---------+---------+---------+ 180
      CCTACAAACCGATATCGTGGTCCATGTTTTTTATATAAAAGTACTTCCTAGTGAGGGAGA

      TATGTAATAGATTTGGGTGAGTGAGTGAGTGAGTGAGTGCATGGACTCACAGCTTTTGGC
  181 ---------+---------+---------+---------+---------+---------+ 240
      ATACATTATCTAAACCCACTCACTCACTCACTCACTCACGTACCTGAGTGTCGAAAACCG

      TTTCTGAAATACCCTGCATCAGTCTTGTTATGATGATTCCTTAGTGCTGGGATGGATCAT
  241 ---------+---------+---------+---------+---------+---------+ 300
      AAAGACTTTATGGGACGTAGTCAGAACAATACTACTAAGGAATCACGACCCTACCTAGTA

      CCAGGCATTTAAGGTAACACGATGGTAATTCTTTGCTCATTTTTCAGGGAAAAAAAAAAG
  301 ---------+---------+---------+---------+---------+---------+ 360
      GGTCCGTAAATTCCATTGTGCTACCATTAAGAAACGAGTAAAAAGTCCCTTTTTTTTTTC

      TTATCACTTCCAAAGTCGGCATAGTCACCCGAAGTAAAAAAAAAAAAAAAAAAAAAAGC
  361 ---------+---------+---------+---------+---------+---------+ 420
      AATAGTGAAGGTTTCAGCCGTATCAGTGGGCTTCATTTTTTTTTTTTTTTTTTTTTTCG

      CTCAGAGGCAAAGGAAAGGGGCCGCAACCTTGGTTAACTGTGAAATGACGAATGAGAAAA
  421 ---------+---------+---------+---------+---------+---------+ 480
      GAGTCTCCGTTTCCTTTCCCCGGCGTTGGAACCAATTGACACTTTACTGCTTACTCTTTT

      CTCCTCCTGCTGAAGATATTCAGGTATATAAAGGCACATGAAGGAAAACTCAAAACATCA
  481 ---------+---------+---------+---------+---------+---------+ 540
      GAGGAGGACGACTTCTATAAGTCCATATATTTCCGTGTACTTCCTTTTGAGTTTTGTAGT

      TTGTCATATACACATCTTCTGGATTTTTTAGCTTGCAAAAAAAATGAGCACCAAACCTGA
  541 ---------+---------+---------+---------+---------+---------+ 600
      AACAGTATATGTGTAGAAGACCTAAAAAATCGAACGTTTTTTTTACTCGTGGTTTGGACT
      → Start
      TATGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTCATTGCTGGCACCCT
  601 ---------+---------+---------+---------+---------+---------+ 660
```

To Page

```
                ATCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGTCACCTGGCAAAATCTGAG
661 ---------+---------+---------+---------+---------+---------+ 720
                TAGGGACCTGACATTGAATGACTTGCAAGTGGACTCTTCTCAGTGGACCGTTTTAGACTC

                ACATCTGAGTAGTATGAGCAATTCATTTCCTGTAGAATGTCTACGAGAAAACATAGCTTT
721 ---------+---------+---------+---------+---------+---------+ 780
                TGTAGACTCATCATACTCGTTAAGTAAAGGACATCTTACAGATGCTCTTTTGTATCGAAA

                TGAGTTGCCCCAAGAGTTTCTGCAATACACCCAACCTATGAAGAGGGACATCAAGAAGGC
781 ---------+---------+---------+---------+---------+---------+ 840
                ACTCAACGGGGTTCTCAAAGACGTTATGTGGGTTGGATACTTCTCCCTGTAGTTCTTCCG

                CTTCTATGAAATGTCCCTACAGGCCTTCAACATCTTCAGCCAACACACCTTCAAATATTG
841 ---------+---------+---------+---------+---------+---------+ 900
                GAAGATACTTTACAGGGATGTCCGGAAGTTGTAGAAGTCGGTTGTGTGGAAGTTTATAAC

                GAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCAGAGTACCTGAA
901 ---------+---------+---------+---------+---------+---------+ 960
                CTTTCTCTCTGTGGAGTTTGTTTAGGTTTATCCTGAACTAGTCGTTCGTCTCATGGACTT

                CCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACATGAAAGAAATGAAAGAGAATGA
961 ---------+---------+---------+---------+---------+---------+ 1020
                GGTTACGAACCTCCTTCTGCTCTTACTTTTACTTCTGTACTTTCTTTACTTTCTCTTACT

                GATGAAACCCTCAGAAGCCAGGGTCCCCCAGCTGAGCAGCCTGGAACTGAGGAGATATTT
1021 ---------+---------+---------+---------+---------+---------+ 1080
                CTACTTTGGGAGTCTTCGGTCCCAGGGGGTCGACTCGTCGGACCTTGACTCCTCTATAAA

                CCACAGGATAGACAATTTCCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGT
1081 ---------+---------+---------+---------+---------+---------+ 1140
                GGTGTCCTATCTGTTAAAGGACTTTCTTTTCTTTATGTCACTGACACGGACCCTCTAACA

                CCGAGTGGAAATCAGAAGATGTTTGTATTACTTTTACAAATTTACAGCTCTATTCAGGAG
1141 ---------+---------+---------+---------+---------+---------+ 1200
                GGCTCACCTTTAGTCTTCTACAAACATAATGAAAATGTTTAAATGTCGAGATAAGTCCTC
                          end
                GAAATAAGGTATATTTTTGGAATTAAAATTCCTTTTCCCTCCGAAATCTCTTTCTCCTTC
1201 ---------+---------+---------+---------+---------+---------+ 1260
                CTTTATTCCATATAAAAACCTTAATTTTAAGGAAAAGGGAGGCTTTAGAGAAAGAGGAAG

                TCCTCCTCCATCTTCTTTTTAAGGATTGTTGTGCTGTCCTGTAAGCCTGTCCTCAGTTGG
1261 ---------+---------+---------+---------+---------+---------+ 1320
                AGGAGGAGGTAGAAGAAAAATTCCTAACAACACGACAGGACATTCGGACAGGAGTCAACC

                ACTGGTAGCCTCGGAACATCAGGGACACTCACCTCTCTAAGGAGAGGTAATGCCAACCAT
1321 ---------+---------+---------+---------+---------+---------+ 1380
                TGACCATCGGAGCCTTGTAGTCCCTGTGAGTGGAGAGATTCCTCTCCATTACGGTTGGTA

                CCTCAGGGTGACCAAGAGTCTCCTTAGAAAGTCTTTAAGACATTTTTAAAGGAATAAGAT
1381 ---------+---------+---------+---------+---------+---------+ 1440
                GGAGTCCCACTGGTTCTCAGAGGAATCTTTCAGAAATTCTGTAAAAATTTCCTTATTCTA

                TCCCTCTCCGTCTTCTTCTATTCTCTCTTGCTCTTTTCTGTGGCCATTTTGAAAGAGCTT
1441 ---------+---------+---------+---------+---------+---------+ 1500
```

To Page No

Date

```
      AGGGAGAGGCAGAAGAAGATAAGAGAGAACGAGAAAAGACACCGGTAAAACTTTCTCGAA

      TGCTATATATACCACCTGTGGACTTCACCAAGACAATGGCTAGAGGATAGGGAGCAGAGA
1501  ---------+---------+---------+---------+---------+---------+ 1560
      ACGATATATATGGTGGACACCTGAAGTGGTTCTGTTACCGATCTCCTATCCCTCGTCTCT

      ATGTTGCAAAATGGTAACATTTCAATGACTTAACTGTTTTGCTGCCAAGGTTGCTTATCC
1561  ---------+---------+---------+---------+---------+---------+ 1620
      TACAACGTTTTACCATTGTAAAGTTACTGAATTGACAAAACGACGGTTCCAACGAATAGG

      TATGAAAATTCAGCACATTAAAAGAGCTTATACATGCTCCCTAGAGTCAATACTCTTGCA
1621  ---------+---------+---------+---------+---------+---------+ 1680
      ATACTTTTAAGTCGTGTAATTTTCTCGAATATGTACGAGGGATCTCAGTTATGAGAACGT

      TTTTCCCCCTCCTGCTCGGGGGGAAAAAGGTTGACATTTCTGGCCCATTTCCTTCTCAGC
1681  ---------+---------+---------+---------+---------+---------+ 1740
      AAAAGGGGGAGGACGAGCCCCCCTTTTTCCAACTGTAAAGACCGGGTAAAGGAAGAGTCG

      TTGGTTTGTTTGAATTGATGCTTGTGGAATGGTATTTCATTACTTTAAGAGTGAAGATCC
1741  ---------+---------+---------+---------+---------+---------+ 1800
      AACCAAACAAACTTAACTACGAACACCTTACCATAAAGTAATGAAATTCTCACTTCTAGG

                                        HindIII
                                           |
      ATAGTGAAATTGGATGGATGGTTGAATTAGACGACCATTAAGCTTGGATCCTCTAGAGCG
1801  ---------+---------+---------+---------+---------+---------+ 1860
      TATCACTTTAACCTACCTACCAACTTAATCTGCTGGTAATTCGAACCTAGGAGATCTCGC

      GCCGCCGACTAGTGAGCTCGTCGACCCGGGAATT
1861  ---------+---------+---------+---- 1894
      CGGCGGCTGATCACTCGAGCAGCTGGGCCCTTAA
```

10 30 50
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70 90 110
CCTAGCAATCAAATAATCACAGTCATTTGGTCAATGTCTATGATTAACTCAATGAGACA
130 150 170
GATGTTTGGCTATAGCACCAGGTACAAAAAATATATTTTCATGAAGGATCACTCCCTCT
190 210 230
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250 270 290
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310 330 350
CAGGCATTTAAGGTAACACGATGGTAATTCTTTGCTCATTTTTCAGGGAAAAAAAAAAG
370 390 410
TATCACTTCCAAAGTCGGCATAGTCACCCGAAGTAAAAAAAAAAAAAAAAAAAAAGC
430 450 470
TCAGAGGCAAAGGAAAGGGGCCGCAACCTTGGTTAACTGTGAAATGACGAATGAGAAAA
490 510 530
TCCTCCTGCTGAAGATATTCAGGTATATAAAGGCACATGAAGGAAAACTCAAAACATCA
550 570 590
TGTCATATACACATCTTCTGGATTTTTTAGCTTGCAAAAAAAATGAGCACCAAACCTGA
610 630 650
ATGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTCATTGCTGGCACCCT
MetIleGlnLysCysLeuTrpLeuGluIleLeuMetGlyIlePheIleAlaGlyThrLe
670 690 710
TCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGTCACCTGGCAAAATCTGAG
SerLeuAspCysAsnLeuLeuAsnValHisLeuArgArgValThrTrpGlnAsnLeuAr
730 750 770
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HisLeuSerSerMetSerAsnSerPheProValGluCysLeuArgGluAsnIleAlaPh
790 810 830
GAGTTGCCCCAAGAGTTTCTGCAATACACCCAACCTATGAAGAGGGACATCAAGAAGGC
GluLeuProGlnGluPheLeuGlnTyrThrGlnProMetLysArgAspIleLysLysAl
850 870 890
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PheTyrGluMetSerLeuGlnAlaPheAsnIlePheSerGlnHisThrPheLysTyrTr
910 930 950
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970 990 1010
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MetLysProSerGluAlaArgValProGlnLeuSerSerLeuGluLeuArgArgTyrPh
1090 1110 1130
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1150 1170 1190
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1210 1230 1250
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ys
1270 1290 1310
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ACTGGTAGCCTCGGAACATCAGGACACTCACCTCTCTTAAGGAGAGGTAATGCAAACCA
1390 1410 1430
CCTCAGGGTGACCAAGAGTCTCCTTAGAAAGTCTTTAAGACATTTTTAAGGAATAAGAT
1450 1470 1490
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1510 1530 1550
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1570 1590 1610
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1630 1650 1670
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1690 1710 1730
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1750 1770 1790
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1810 1830 1850
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To Page No.

pst clone

```
          10                  30                  50
GTCGACCCACGCGTCCGGGGTGTTGTAGATATTTTTCCTTTGGAAGAAATACTGAGCACC
          70                  90                 110
AAGGCTGAGATGACACTGAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTTCA
            MetThrLeuLysTyrLeuTrpLeuValAlaLeuValAlaLeuTyrIleSer
         130                 150                 170
CCCATCCAGTCTCAGAACTGTGTGTATCTGGATCATACCATCTTGGAAAACATGAAACTT
ProIleGlnSerGlnAsnCysValTyrLeuAspHisThrIleLeuGluAsnMetLysLeu
         190                 210                 230
CTGAGCAGCATCAGGACCACCTTTCCCTTAAGATGTCTAAAAGATATCACGGATTTTGAG
LeuSerSerIleArgThrThrPheProLeuArgCysLeuLysAspIleThrAspPheGlu
         250                 270                 290
TTTCCTCAAGAGATTCTGCTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACC
PheProGlnGluIleLeuLeuTyrValGlnHisValLysLysAspIleLysAlaValThr
         310                 330                 350
TATCATATATCTTCTCTGGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGGCG
TyrHisIleSerSerLeuAlaLeuIleIlePheSerLeuLysAspSerIleSerLeuAla
         370                 390                 410
ACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTCAAACAAGTGCAGCAAGCTCGA
ThrGluGluArgLeuGluArgIleArgSerGlyLeuPheLysGlnValGlnGlnAlaArg
         430                 450                 470
GAGTGCATGGTAGACGAGGAGAACAAGAACACGGAGGAGGACAGTACATCACAACATCCT
GluCysMetValAspGluGluAsnLysAsnThrGluGluAspSerThrSerGlnHisPro
         490                 510                 530
CACTCAGAGGGCTTCAAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGA
HisSerGluGlyPheLysAlaValTyrLeuGluLeuAsnLysTyrPhePheArgIleArg
         550                 570                 590
AAGTTCCTGGTAAATAAGAAATACAGTTTCTGTGCCTGGAAGATTGTCGTGGTGGAAATA
LysPheLeuValAsnLysLysTyrSerPheCysAlaTrpLysIleValValValGluIle
         610                 630                 650
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ArgArgCysPheSerIlePheTyrLysLeuLeuAsnMetAsnEnd
         670                 690                 710
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         730                 750                 770
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         790                 810                 830
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         850                 870                 890
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         910                 930                 950
CAAAAAAAATAAAACAAAAAAAAACAACAGTCACTAAAAAAAAAAAAAAAAAAGGGCGGC

CGC
```

To Page

Nucleotide comparison

JHN

6

WChen
DNA=hgmrep3-6.2
assembled by JK[redacted]
9808903.con

to: Mrpe3-00078-F6-Wz.Ctg check: 4485 from: 1 to: 963

From: INTRON::DONGYINY [redacted] 16:07:43.44
To: WCHEN
CC:
Subj:
From: INTRON::JCAO [redacted] 12:24:38.19
To: DONGYINY . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Swgapdna.Cmp
CompCheck: 2335

Gap Weight:	50	Average Match:	10.000
Length Weight:	3	Average Mismatch:	-9.000
Quality:	1457	Length:	634
Ratio:	2.392	Gaps:	6
Percent Similarity:	63.516	Percent Identity:	63.516

Match display thresholds for the alignment(s):
| = IDENTITY
: = 5
. = 1

9808903.Rev x Mrpe3-00078-F6-Wz.Ctg [redacted] 15:04 ..

```
555 TCTTCTGGATTTTTTAGCTT..GCAAAAAAAATGAGCACCAAACCTGATA 602
    | || | ||| ||||  |||  | | ||| | ||||||||||  |||| |
 21 TGTTGTAGATATTTTTCCTTTGGAAGAAATACTGAGCACCAAGGCTGAGA 70

603 TGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTCATTGCT 652
    |||  |  ||| ||| ||||| | |. ||| ||| | ||| |||| |
 71 TGACACTGAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTTCA 120

653 GGCACCCTATCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGT 702
      || ||  || | | |||||            ||  | |||
121 CCCATCCAGTCTCAGAACTGT.............GTGTATCTGGATCATAC 158

703 CACCTGGCAAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCTG 752
    || || | ||||  ||| || |||||| || || || |   | |||||
159 CATCTTGGAAAACATGAAACTTCTGAGCAGCATCAGGACCACCTTTCCCT 208

753 TAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCCAAGAGTTTCTG 802
    ||  ||||||  ||| | ||  | ||||||||| || |||||| |||||
209 TAAGATGTCTAAAAGATATCACGGATTTTGAGTTTCCTCAAGAGATTCTG 258

803 CAATACACCCAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAAT 852
    |  |||  ||| | | |||| | |||||| |||   | |  |||| | ||
259 CTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACCTATCATAT 308
```

To Page N

```
853 GTCCCTACAGGCCTTCAACATCTTCAGCC...AACACACCTTCAAATATT 899
309 ATCTTCTCTGGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGG 358

900 GGAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCA 949
359 CGACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTCAAACAAGTG 408

950 GAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACAT 999
409 CAGCAAGCTCGAGAGTGCATGGTAGACGAGGAGAACAAGA......ACAC 452

1000 GAAAGAAATGAAAGAGAATGAGATGAAACCCTCAGAAGCCAG.GGTCCCC 1048
453 GGAGG.......AGGACAGTACATCACAACATCCTCACTCAGAGGGCTTC 495

1049 CAGCTGAGCAGCCTGGAACTGAGGAGATATTTCCACAGGATAGACAATTT 1098
496 AAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGAAAGTT 545

1099 CCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGTCCGAGTGG 1148
546 CCTGGTAAATAAGAAATACAGTTTCTGTGCCTGGAAGATTGTCGTGGTGG 595

1149 AAATCAGAAGATGTTTGTATTACTTTTACAAATT 1182
596 AAATAAGAAGATGTTTCAGTATATTTTACAAACT 629
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IFN

protein sequence comp

BESTFIT of: 9808903.Pep check: 4904 from: 1 to: 201

TRANSLATE of: 9808903.rev check: 8672 from: 602 to: 1205
generated symbols 1 to: 201.
REVERSE-COMPLEMENT of: 9808903.Con check: 57 from: 1 to: 1894
WChen
DNA=hgmrep3-6.2
assembled by JK: [redacted] . . .

to: Mrpe3-00078-F6-Wz.Pep check: 5990 from: 1 to: 192

TRANSLATE of: mrpe3-00078-f6-wz.ctg check: 4485 from: 70 to: 646
generated symbols 1 to: 192.
From: INTRON::DONGYINY [redacted] 16:07:43.44
To: WCHEN
CC:
Subj: . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Blosum62.Cmp
CompCheck: 6430

Gap Weight:	12	Average Match:	2.912
Length Weight:	4	Average Mismatch:	-2.003
Quality:	292	Length:	194
Ratio:	1.570	Gaps:	3
Percent Similarity:	49.730	Percent Identity:	40.541

Match display thresholds for the alignment(s):
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: = 2
. = 1

9808903.Pep x Mrpe3-00078-F6-Wz.Pep [redacted] 14:55 ..

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        |  | |||  |. ::|.   | .|    |:|     :|:: |||.  .||
      1 MILKYLWLVALVALYISPIQSQNC....VYLDHTILENMKLLSSIRTTFP 46

     51 VECLRENIAFELPQEFLQYTQPMKRDIKKAFYEMSLQAFNIFS.QHTFKY 99
        . ||::   || ||| | | | .|:|||   | .|  |  ||| . .
     47 LRCLKDITDFEFPQEILLYVQHVKKDIKAVTYHISSLALIIFSLKDSISL 96

    100 WKERHLKQIQIGLDQQAEYLNQCLEEDENENEDMKEMKENEMKPSEARVP 149
          |  |..|. || .| :   :|: ::||.| :     :.       |
     97 ATEERLERIRSGLFKQVQQARECMVDEENKNIEEDSTSQHPHSEGFKAV. 145

    150 QLSSLELRRYFHRIDNFLKEKKYSDCAWEIVRVEIRRCLYYFYK 193
          ||| :|| ||  ||  |||| |||.|| |||||   |||
    146 ...YLELNKYFFRIRKFLVNKKYSFCAWKIVVVEIRRCFSIFYK 186
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tnessed & Understood by me, | Date | Invented by | Date

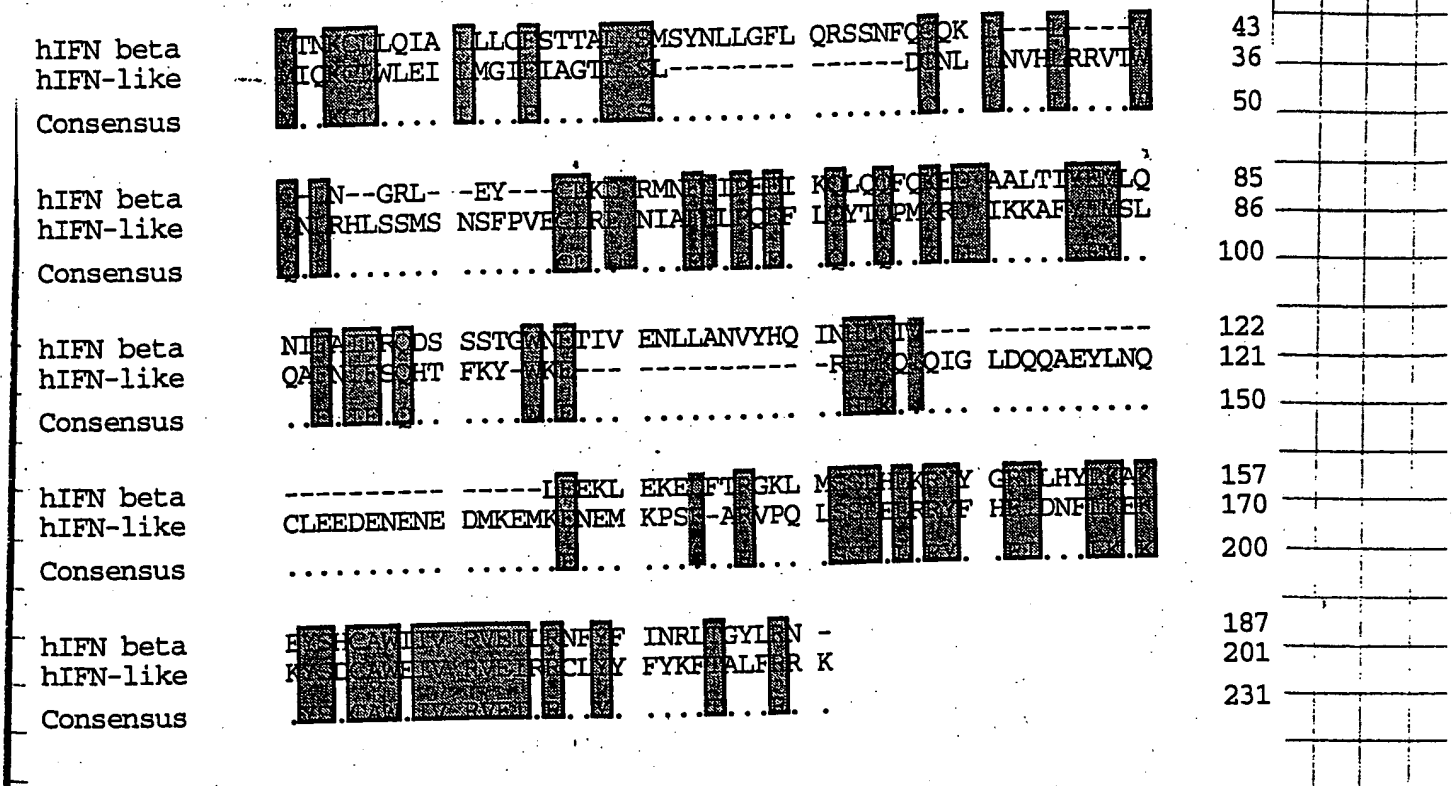
Project No._____

Book No._____ TITLE _____

IFN

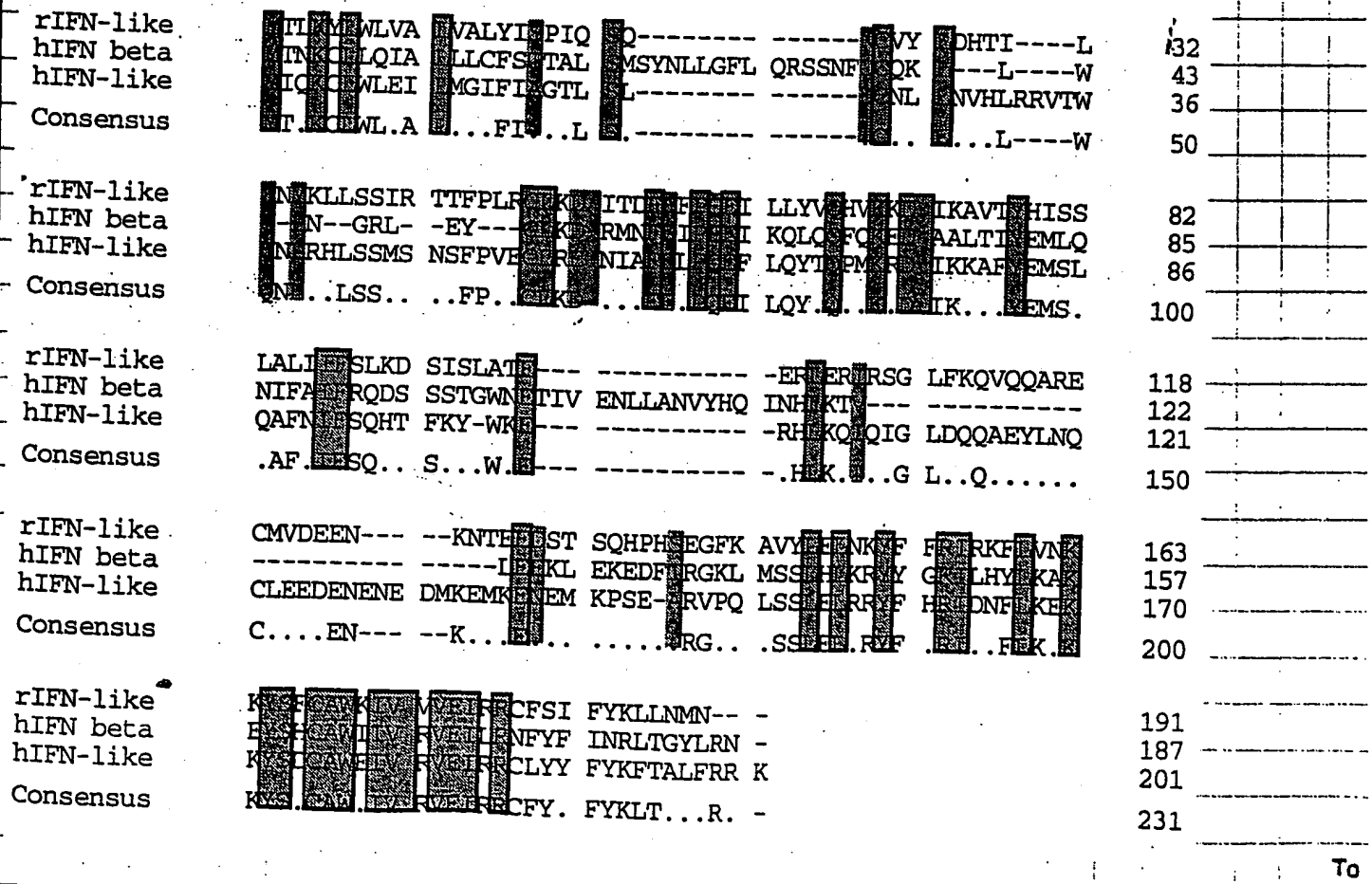
Untitled-5 Formatted Alignment

27% identity



Untitled-5 Formatted Alignment

17% iden



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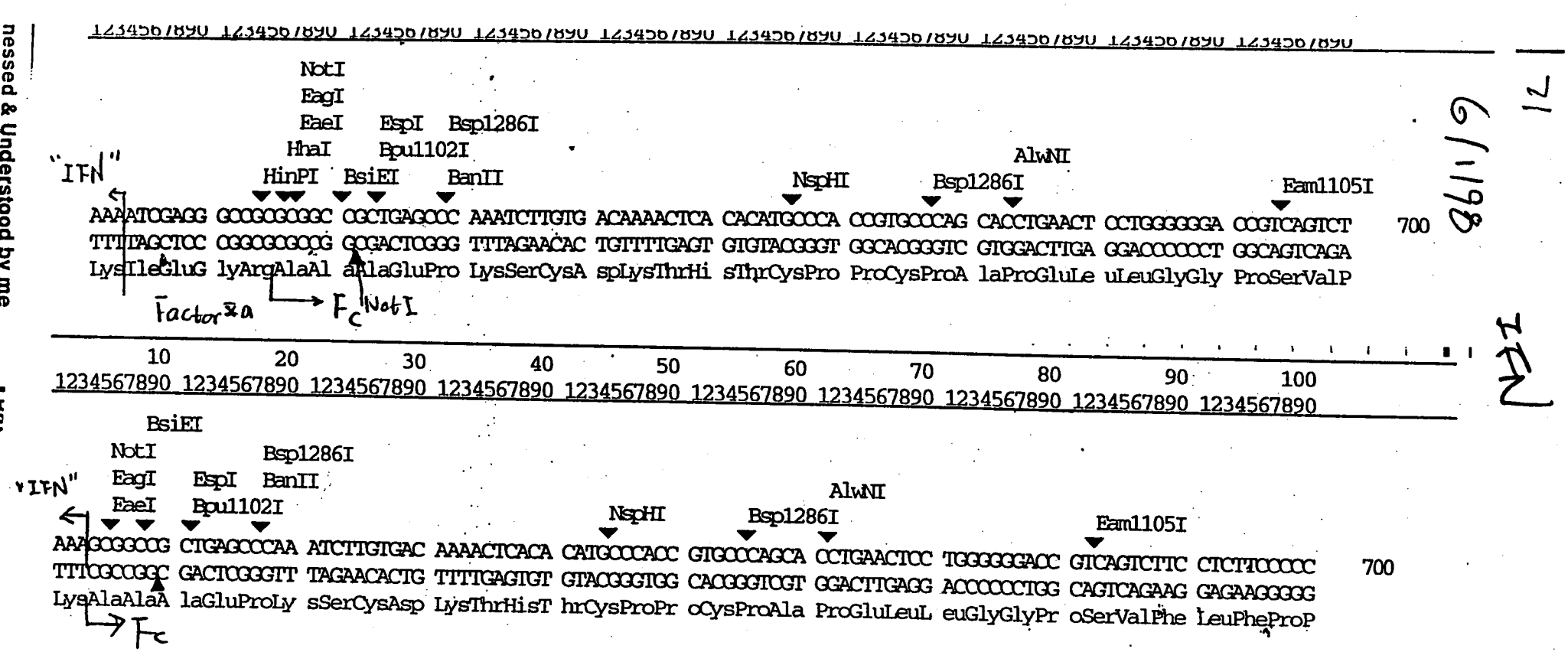

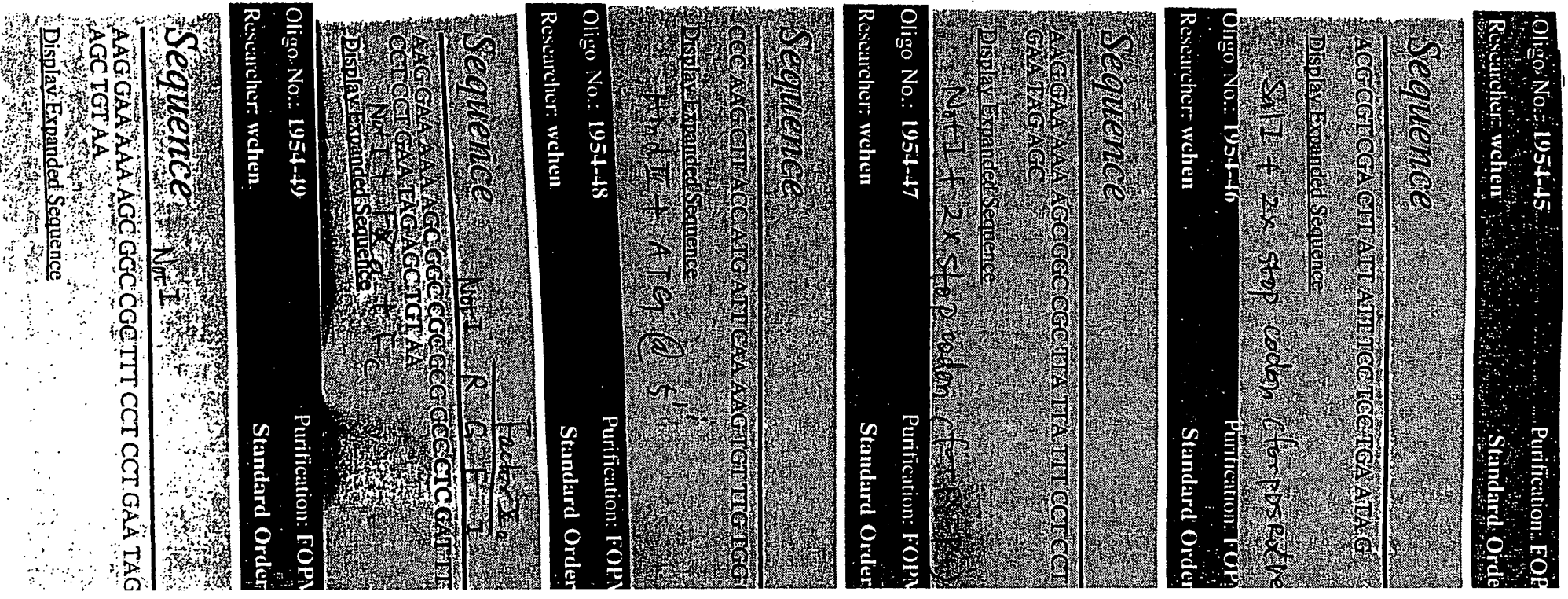

From Page No.___

Chen, Wen

From: Schultz, Henry
Sent: Friday, [redacted] 5:15 PM
To: Chen, Wen
Subject: RE: human interferon like seq.

Wen - the human predicts cytokine strongly
scoreaacomp= -2.9 scoredipep= 19 ACCEPT (Probablility 91%).

The human is predicted to be signal peptide as follows:

MIQKCLWLEILMGIFIAGTLS cleavage LD.....etc

For the rat, the cytokine prediction is lost but the signal peptide is:

MTLKYLWLVALVALYISPIQS cleavage QN....etc

Henry

From: Chen, Wen
Sent: Friday, [redacted] 4:02 PM
To: Schultz, Henry
Subject: human interferon like seq.

Henry: please help me look at the signal peptide for the human clone sequence.
Thanks.

Wen Chen

Human sequence:
MIQKCLWLEI LMGIFIAGTL SLDCNLLNVH LRRVTWQNLR HLSSMSNSFP

51 VECLRENIAF ELPQEFLQYT QPMKRDIKKA FYEMSLQAFN IFSQHTFKYW

101 KERHLKQIQI GLDQQAEYLN QCLEEDENEN EDMKEMKENE MKPSEARVPQ

151 LSSLELRRYF HRIDNFLKEK KYSDCAWEIV RVEIRRCLYY FYKFTALFRR

201 K

[redacted]

Project No.________

Book No.________

```
        10                           30                           50
CGCGTACGTAAGCTTAATTTAACAAAATTGGAAAAACCTAAACTATACTGTGCTCTGGTG
        70                           90                           110
ACCTAGCAATCAAATAATCACAGTCATTTGGTCAATGTCTATGATTAACTCAATGAGACA
        130                          150                          170
GGATGTTTGGCTATAGCACCAGGTACAAAAAATATATTTTCATGAAGGATCACTCCCTCT
        190                          210                          230
TATGTAATAGATTTGGGTGAGTGAGTGAGTGAGTGAGTGCATGGACTCACAGCTTTTGGC
        250                          270                          290
TTTCTGAAATACCCTGCATCAGTCTTGTTATGATGATTCCTTAGTGCTGGGATGGATCAT
        310                          330                          350
CCAGGCATTTAAGGTAACACGATGGTAATTCTTTGCTCATTTTTCAGGGAAAAAAAAAAG
        370                          390                          410
TTATCACTTCCAAAGTCGGCATAGTCACCCGAAGTAAAAAAAAAAAAAAAAAAAAAAAGC
        430                          450                          470
CTCAGAGGCAAAGGAAAGGGGCCGCAACCTTGGTTAACTGTGAAATGACGAATGAGAAAA
        490                          510                          530
CTCCTCCTGCTGAAGATATTCAGGTATATAAAGGCACATGAAGGAAAACTCAAAACATCA
        550                          570                          590
TTGTCATATACACATCTTCTGGATTTTTTAGCTTGCAAAAAAAATGAGCACCAAACCTGA
                                             MetSerThrLysProAs
        610                          630                          650
TATGATTCAAAAGTGTTTGTGGCTTGAGATCCTTATGGGTATATTCATTGCTGGCACCCT
pMetIleGlnLysCysLeuTrpLeuGluIleLeuMetGlyIlePheIleAlaGlyThrLe
        670                          690                          710
ATCCCTGGACTGTAACTTACTGAACGTTCACCTGAGAAGAGTCACCTGGCAAAATCTGAG
uSerLeuAspCysAsnLeuLeuAsnValHisLeuArgArgValThrTrpGlnAsnLeuAr
        730                          750                          770
ACATCTGAGTAGTATGAGCAATTCATTTCCTGTAGAATGTCTACGAGAAAACATAGCTTT
gHisLeuSerSerMetSerAsnSerPheProValGluCysLeuArgGluAsnIleAlaPh
        790                          810                          830
TGAGTTGCCCCAAGAGTTTCTGCAATACACCCAACCTATGAAGAGGGACATCAAGAAGGC
eGluLeuProGlnGluPheLeuGlnTyrThrGlnProMetLysArgAspIleLysLysAl
        850                          870                          890
CTTCTATGAAATGTCCCTACAGGCCTTCAACATCTTCAGCCAACACACCTTCAAATATTG
aPheTyrGluMetSerLeuGlnAlaPheAsnIlePheSerGlnHisThrPheLysTyrTr
        910                          930                          950
GAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCAGAGTACCTGAA
pLysGluArgHisLeuLysGlnIleGlnIleGlyLeuAspGlnGlnAlaGluTyrLeuAs
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        1030                         1050                         1070
GATGAAACCCTCAGAAGCCAGGGTCCCCCAGCTGAGCAGCCTGGAACTGAGGAGATATTT
uMetLysProSerGluAlaArgValProGlnLeuSerSerLeuGluLeuArgArgTyrPh
        1090                         1110                         1130
CCACAGGATAGACAATTTCCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGT
eHisArgIleAspAsnPheLeuLysGluLysLysTyrSerAspCysAlaTrpGluIleVa
        1150                         1170                         1190
CCGAGTGGAAATCAGAAGATGTTTGTATTACTTTTACAAATTTACAGCTCTATTCAGGAG
lArgValGluIleArgArgCysLeuTyrTyrPheTyrLysPheThrAlaLeuPheArgAr
        1210                         1230                         1250
GAAATAAGGTATATTTTTGGAATTAAAATTCCTTTTCCCTCCGAAATCTCTTTCTCCTTC
```

To Page No.______

Date

Project No._____
Book No._____ TITLE IFN

From Page No.___

gLysEnd

1270 1290 1310
TCCTCCTCCATCTTCTTTTTAAGGATTGTTGTGCTGTCCTGTAAGCCTGTCCTCAGTTGG
1330 1350 1370
ACTGGTAGCCTCGGAACATCAGGGACACTCACCTCTCTAAGGAGAGGTAATGCCAACCAT
1390 1410 1430
CCTCAGGGTGACCAAGAGTCTCCTTAGAAAGTCTTTAAGACATTTTTAAAGGAATAAGAT
1450 1470 1490
TCCCTCTCCGTCTTCTTCTATTCTCTCTTGCTCTTTTCTGTGGCCATTTTGAAAGAGCTT
1510 1530 1550
TGCTATATATACCACCTGTGGACTTCACCAAGACAATGGCTAGAGGATAGGGAGCAGAGA
1570 1590 1610
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1630 1650 1670
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1750 1770 1790
TTGGTTTGTTTGAATTGATGCTTGTGGAATGGTATTTCATTACTTTAAGAGTGAAGATCC
1810 1830 1850
ATAGTGAAATTGGATGGATGGTTGAATTAGACGACCATTAAGCTTGGATCCTCTAGAGCG
1870 1890
GCCGCCGACTAGTGAGCTCGTCGACCCGGGAATT

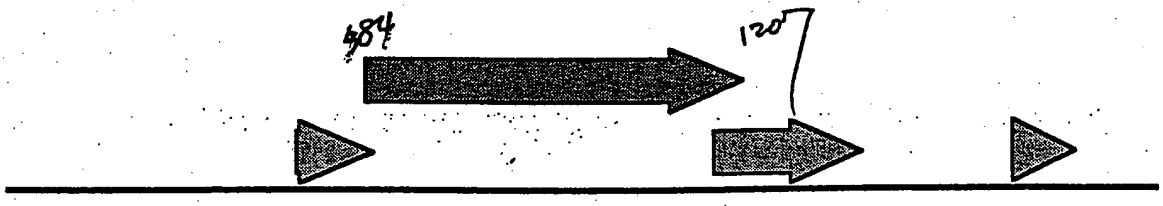
0 400 800 1200 1600 2000

ORF Map (Universal)

Frame 3

Frame 2

Frame 1



Frame -1

Frame -2

Frame -3

One more ATG @ 584 in frame with ATG starting from 602.

To Page N

Witnessed & Understood by me. Date Invented by Date

D 1067 McDonnell GN-0001

RESEARCH SUMMARY PAGE

Gene Name:	**All Known Alias Gene Names:**
Human: Zhwxc00-00001-a1 Rat: Agp-22423-a1	Member of the interferon family of proteins Name: Interferon-like protein.

Investigator(s):	**Initial Date of Summary Preparation:**
Duanzhi Wen, Andrew Welcher, Michael Kelley	Initial invention disclosure filed [redacted] This summary filled in on [redacted]

Description of Project:

This novel member of the interferon family of proteins is related to the beta, alpha, and omega subfamilies. As an interferon it would be expected to have anti-infective and anti-proliferative uses. Additionally, it might find use in the treatment of multiple sclerosis and other pathologies requiring immunomodulation.

Gene Nucleotide Sequence:

Human:

```
1    CGCGTACGTA AGCTTAATTT AACAAAATTG GAAAAACCTA AACTATACTG
51   TGCTCTGGTG ACCTAGCAAT CAAATAATCA CAGTCATTTG GTCAATGTCT
101  ATGATTAACT CAATGAGACA GGATGTTTGG CTATAGCACC AGGTACAAAA
151  AATATATTTT CATGAAGGAT CACTCCCTCT TATGTAATAG ATTTGGGTGA
201  GTGAGTGAGT GAGTGAGTGC ATGGACTCAC AGCTTTTGGC TTTCTGAAAT
251  ACCCTGCATC AGTCTTGTTA TGATGATTCC TTAGTGCTGG GATGGATCAT
301  CCAGGCATTT AAGGTAACAC GATGGTAATT CTTTGCTCAT TTTTCAGGGA
351  AAAAAAAAAG TTATCACTTC CAAAGTCGGC ATAGTCACCC GAAGTAAAAA
401  AAAAAAAAAA AAAAAAAAGC CTCAGAGGCA AAGGAAAGGG GCCGCAACCT
451  TGGTTAACTG TGAAATGACG AATGAGAAAA CTCCTCCTGC TGAAGATATT
501  CAGGTATATA AAGGCACATG AAGGAAAACT CAAAACATCA TTGTCATATA
551  CACATCTTCT GGATTTTTTA GCTTGCAAAA AAAATGAGCA CCAAACCTGA
601  TATGATTCAA AAGTGTTTGT GGCTTGAGAT CCTTATGGGT ATATTCATTG
651  CTGGCACCCT ATCCCTGGAC TGTAACTTAC TGAACGTTCA CCTGAGAAGA
701  GTCACCTGGC AAAATCTGAG ACATCTGAGT AGTATGAGCA ATTCATTTCC
751  TGTAGAATGT CTACGAGAAA ACATAGCTTT TGAGTTGCCC CAAGAGTTTC
801  TGCAATACAC CCAACCTATG AAGAGGGACA TCAAGAAGGC CTTCTATGAA
851  ATGTCCCTAC AGGCCTTCAA CATCTTCAGC CAACACACCT TCAAATATTG
901  GAAAGAGAGA CACCTCAAAC AAATCCAAAT AGGACTTGAT CAGCAAGCAG
951  AGTACCTGAA CCAATGCTTG GAGGAAGACG AGAATGAAAA TGAAGACATG
1001  AAAGAAATGA AAGAGAATGA GATGAAACCC TCAGAAGCCA GGGTCCCCCA
1051  GCTGAGCAGC CTGGAACTGA GGAGATATTT CCACAGGATA GACAATTTCC
1101  TGAAAGAAAA GAAATACAGT GACTGTGCCT GGGAGATTGT CCGAGTGGAA
1151  ATCAGAAGAT GTTTGTATTA CTTTTACAAA TTTACAGCTC TATTCAGGAG
1201  GAAATAAGGT ATATTTTTGG AATTAAAATT CCTTTTCCCT CCGAAATCTC
1251  TTTCTCCTTC TCCTCCTCCA TCTTCTTTTT AAGGATTGTT GTGCTGTCCT
1301  GTAAGCCTGT CCTCAGTTGG ACTGGTAGCC TCGGAACATC AGGGACACTC
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1351  ACCTCTCTAA GGAGAGGTAA TGCCAACCAT CCTCAGGGTG ACCAAGAGTC
1401  TCCTTAGAAA GTCTTTAAGA CATTTTTAAA GGAATAAGAT TCCCTCTCCG
1451  TCTTCTTCTA TTCTCTCTTG CTCTTTTCTG TGGCCATTTT GAAAGAGCTT
1501  TGCTATATAT ACCACCTGTG GACTTCACCA AGACAATGGC TAGAGGATAG
1551  GGAGCAGAGA ATGTTGCAAA ATGGTAACAT TTCAATGACT TAACTGTTTT
1601  GCTGCCAAGG TTGCTTATCC TATGAAAATT CAGCACATTA AAAGAGCTTA
1651  TACATGCTCC CTAGAGTCAA TACTCTTGCA TTTTCCCCCT CCTGCTCGGG
1701  GGGAAAAAGG TTGACATTTC TGGCCCATTT CCTTCTCAGC TTGGTTTGTT
1751  TGAATTGATG CTTGTGGAAT GGTATTTCAT TACTTTAAGA GTGAAGATCC
1801  ATAGTGAAAT TGGATGGATG GTTGAATTAG ACGACCATTA AGCTTGGATC
1851  CTCTAGAGCG GCCGCCGACT AGTGAGCTCG TCGACCCGGG AATT
```

Rat:

```
   1 GGGTGTTGTA GATATTTTTC CTTTGGAAGA AATACTGAGC ACCAAGGCTG
  51 AGATGACACT GAAGTATTTA TGGCTGGTGG CCCTCGTGGC TCTATACATT
 101 TCACCCATCC AGTCTCAGAA CTGTGTGTAT CTGGATCATA CCATCTTGGA
 151 AAACATGAAA CTTCTGAGCA GCATCAGGAC CACCTTTCCC TTAAGATGTC
 201 TAAAAGATAT CACGGATTTT GAGTTTCCTC AAGAGATTCT GCTGTACGTC
 251 CAGCATGTGA AAAAGGACAT AAAGGCAGTC ACCTATCATA TATCTTCTCT
 301 GGCGCTAATT ATTTTCAGTC TTAAAGACTC CATCTCCCTG GCGACAGAGG
 351 AACGCTTGGA ACGTATCAGA TCGGGACTTT TCAAACAAGT GCAGCAAGCT
 401 CGAGAGTGCA TGGTAGACGA GGAGAACAAG AACACGGAGG AGGACAGTAC
 451 ATCACAACAT CCTCACTCAG AGGGCTTCAA GGCAGTCTAC CTGGAATTGA
 501 ACAAGTATTT CTTCAGAATC AGAAAGTTCC TGGTAAATAA GAAATACAGT
 551 TTCTGTGCCT GGAAGATTGT CGTGGTGGAA ATAAGAAGAT GTTTCAGTAT
 601 ATTTTACAAA CTACTCAACA TGAATTGAGA ATCATCCAGC TTCAAGCAAG
 651 AACTTAGATA GAAGTTGTGA CTGCTCAAAT GTCCCCAAGA ACGCTTGATT
 701 CTAAGGCTAT TGCGAGTCTG CTGCTACACA CTTCGGACGC AAGACTTTTC
 751 AAGGTCAGGG TTCAAGGTAG TACAGTCAAA GGAAGTCTTA TGTTAAGCAA
 801 AAGAAAAATT TCAGTGGAAA AGCTAGCAGA AATGTCAACT TGTCAAAAAA
 851 ACAACTTATG GATTATGGCA TTGACGTTAC TAGCAAAAAA AATAAAACAA
 901 AAAAAAACAA AAA
```

Gene Amino Acid Sequence:

Human:

```
1   MSTKPDMIQK CLWLEILMGI FIAGTLSLDC NLLNVHLRRV TWQNLRHLSS
51   MSNSFPVECL RENIAFELPQ EFLQYTQPMK RDIKKAFYEM SLQAFNIFSQ
101   HTFKYWKERH LKQIQIGLDQ QAEYLNQCLE EDENENEDMK EMKENEMKPS
151   EARVPQLSSL ELRRYFHRID NFLKEKKYSD CAWEIVRVEI RRCLYYFYKF
201   TALFRRK*
```

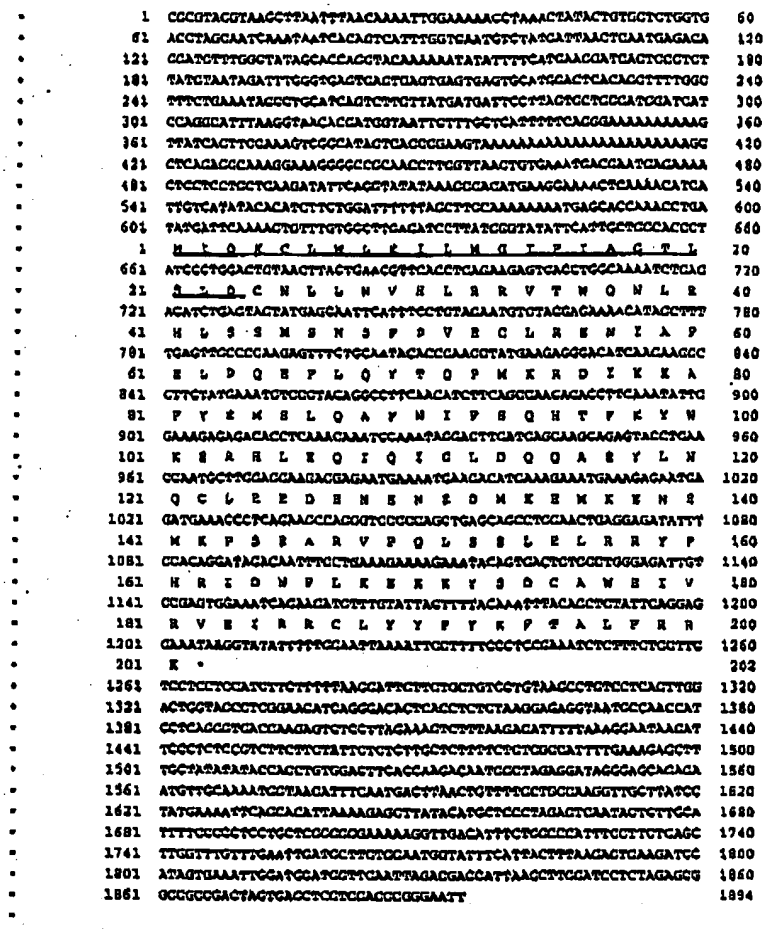
Rat:

```
1   MTLKYLWLVA LVALYISPIQ SQNCVYLDHT ILENMKLLSS IRTTFPLRCL
51   KDITDFEFPQ EILLYVQHVK KDIKAVTYHI SSLALIIFSL KDSISLATEE
101   RLERIRSGLF KQVQQARECM VDEENKNTEE DSTSQHPHSE GFKAVYLELN
151   KYFFRIRKFL VNKKYSFCAW KIVVVEIRRC FSIFYKLLNM N*
```

Figure Containing cDNA and Amino Acid Sequences:

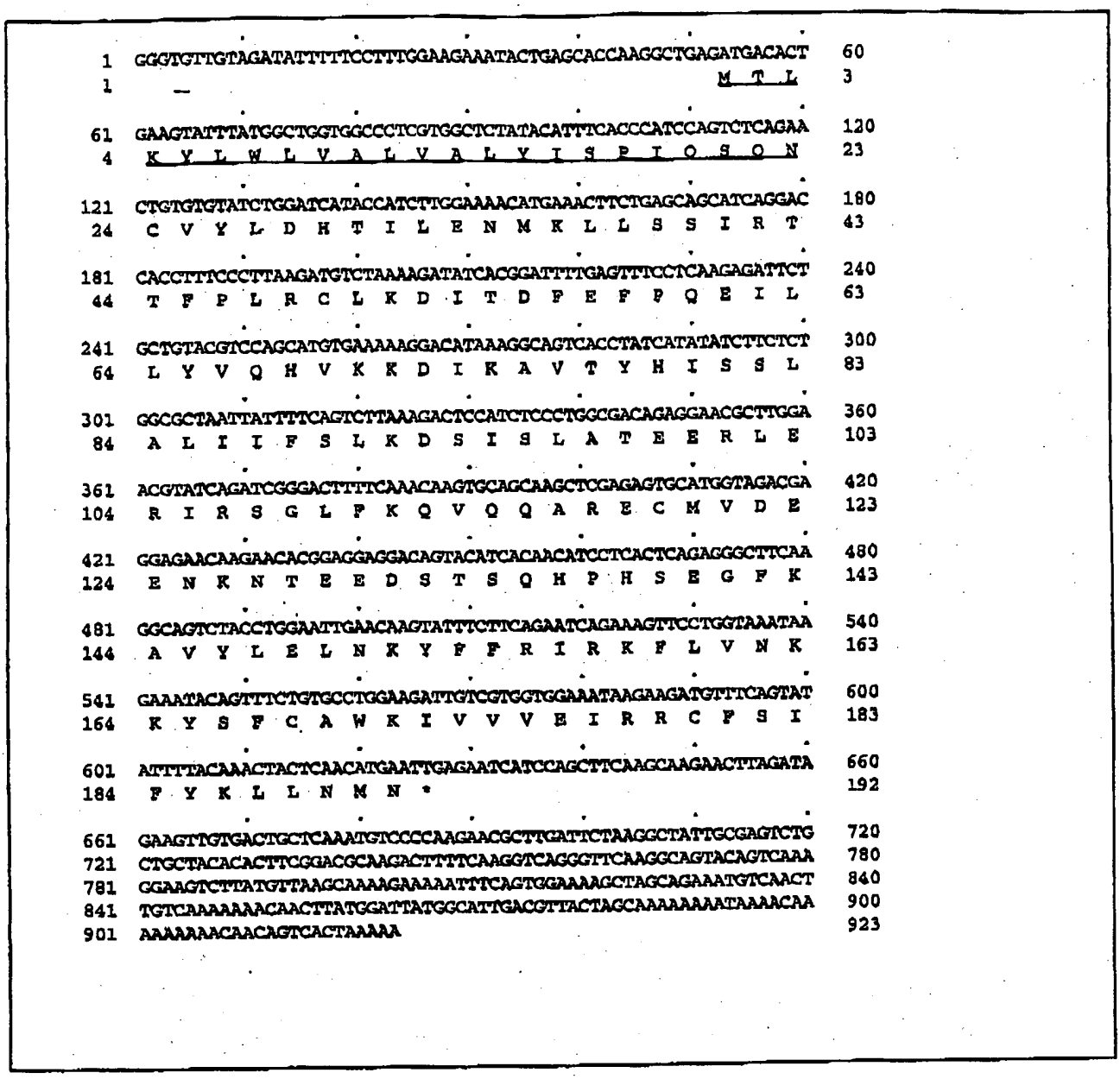
Human:

Sequence Analysis of Human IFN-novel



A human gene which encodes a novel protein of 207 amino acids was isolated by screening the human genomic DNA library using a rat cDNA clone. The deduced amino acid sequence of this novel gene is indicated below the first nucleotide of each codon, and the termination codon is marked with an asterisk. The protein starts with cysteine, and the signal peptide is underlined. This novel protein is 27% identical to human IFN-β.

Rat:



Cloning Information:

The rat sequence was cloned from a rat placenta cDNA library as part of an EST project and was identified by computer analysis as being a novel member of the interferon family of proteins. Briefly, rat embryo day 17 [E17] placenta mRNA was isolated by standard methods (unnecessary information) (Chomczynski, P. and Sacchi, N., Anal. Biochem. 162, 156, 1987). cDNA was synthesized using the SuperScript Plasmid cDNA kit supplied by GIBCO/BRL and subcloned into the pSPORT1 (GIBCO/BRL) vector into the Sal 1 and Not 1 restriction sites.

Cloning of Human IFN-like gene:

Multiple attempts to clone the human IFN-like gene from a variety of human tissue cDNA libraries failed to yield positive clones. However, a human tissue Northern Blot

hybridized with a PCR-generated radioactive rat probe revealed an 1.8 kb Hind III fragment in certain batches of human pancreas mRNA. Attempts to clone this corresponding message in a pancreas cDNA library failed to recover any positive clones.

Examination of the genomic structures of known IFNs revealed that IFN, especially the members in the IFNa family, all share a unique intronless genomic structure. Therefore, screening of human genomic DNA might yield the complete human IFN-like gene. We started with $1x10^6$ human lambda genomic clones (Stratagene, Cat. No. 946206) for primary screening at a density of 50, 000 clonies / plate (*unnecessary information*). Nitrocellulose filters (*unnecessary information*) (S&S) were prepared by standard techniques (Molecular Cloning, A Laboratory Manual, Sambrook, Fritsch, and Maniatis editors).

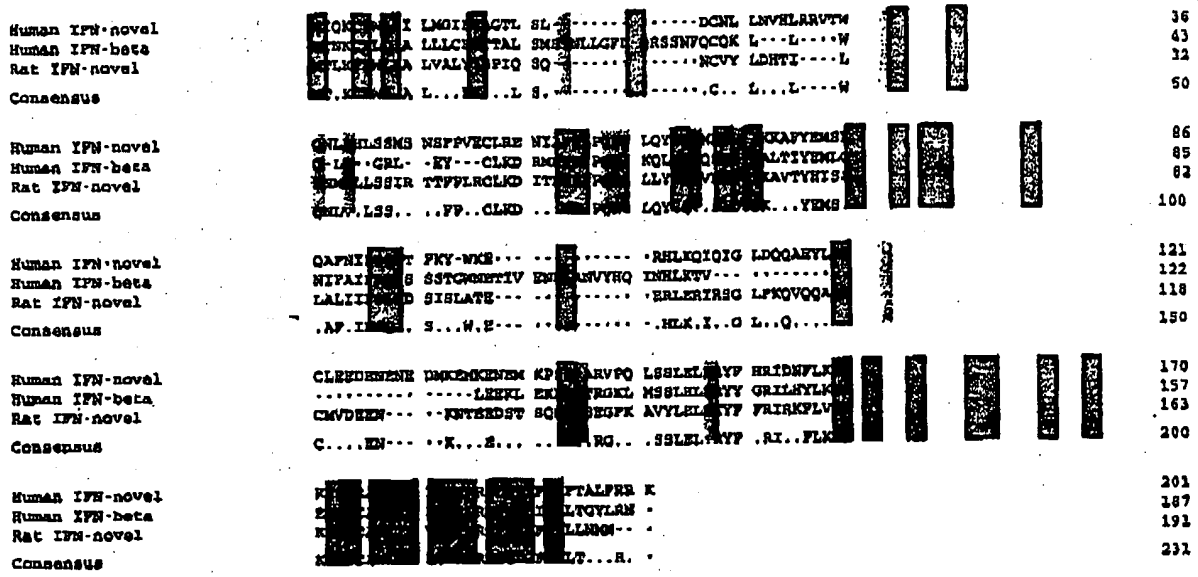
The following conditions were used.

- Prehybridization and hybridization conditions: 30% formamide, 5x SSC, 2x Denhart's, 10μg/ml Salmon sperm DNA, 0.2% SDS, 2mM EDTA and 0.1% pyrophosphate. Hybridization was conducted overnight at 42°C. The washings were done under following conditions: 1x SSC, 0.1%SDS at room temperature for 30-60 minutes followed by 0.2x SSC and 0.1%SDS at 55°C for 15 minutes.
- Generation of radioactive PCR probe (*unnecessary information*): rat cDNA full-length fragment 20ng, primer 1795-01 and 1795-02, 20 pmol each, 1mmol dNTP (dCTP @ 0.01mmol), 32P-dCTP 5 ml and 4mM MgCl2. Reaction condition: denature at 94 °C for 30sec, anneal at 60 °C for 30sec and elongate at 72 °C for 1 minute. The reaction is repeated for a total of 45 times. Simultaneously a "cold" PCR reaction is performed under exact condition except the dNTP mix is dCTP balanced. The radioactive probe was purified by Quick Spin G-50 column and boiled at 100 °C for 10 minutes before chilling on dry ice for 20 minutes. The probe is usually $5x10^5$ cpm/μl.

Three positive clones were recovered after primary, secondary screening and subsequently purified to homogeneity. The lambda phage DNA was prepared by a solid plate culture method. The NotI insert from these clones were excised out and ligated into pSport (GIBCO BRL) vector and transformed into DH10 E. coli strain. The transformants were prepared by Qiagen Spin Column plasmid prep kit. The plasmid DNA was then digested with HindIII. The digested fragments were resolved on agarose gel and transferred to a nylon membrane for Southern Blot analysis. The analysis was conducted under the same condition genomic screening was carried out. The corresponding fragment recognized by "hot" rat probe was then subloned in pSport vector for sequencing analysis. According to the HindIII digestion pattern, we determined these three independent clones were likely to contain identical genomic insert. The sequencing analysis confirmed our speculation. This 1.8kb HindIII fragment contains an open reading frame of 624 base pairs that has 64% similarity to the sequence of rat mrpe3-00078-F6-Wz. In terms of similarity in amino acid sequence, the human sequence is 40.5% identical to and 50% similar to that of rat. All 5 predicted Cysteine residues were perfectly aligned with those in rat protein sequence. Moreover, the human sequence is predicted to contain a signal peptide and cleavage site. The human IFN-like protein is strongly predicted to resemble a secreted cytokine molecule (91% probability).

Homology of Multiple Gene Family Members:

Amino Acid Sequence Alignment of Human IFN-novel, Rat IFN-novel and Human IFN-β



- Human IFN-novel is most close to human IFN-β, with 30% identity. Four out of five cysteine residues are conserved between them.

Presence and Distribution of mRNA in Different Tissues:

Northern blot analysis detected IFN-like mRNA in several different stages of mouse and rat embryos. Northern blots used RNA isolated as above. The full-length rat cDNA was used as a probe. Prehyb conditions were 40 % formamide, 5X SSC, 1 mM EDTA, 0.1 % SDS, for 4 h at 42°C. Hyb conditions were the same as above except were done overnight at 42°C. Blots were washed with 0.2x SSC, 1 mM EDTA, and 0.1% SDS for 30 min at 60°C.

RT-PCR *(conditions are not necessary - standard technology)* identified IFN-like mRNA in the following human tissues : pancreas, small intestine, prostate, uterus, thyroid, and placenta.

Recombinant Protein Expression:

Production of human and rat IFN-like protein in E. coli :

Waiting on data from Karen Sitney. However, the E. coli protein did not appear to be folded correctly and has not yet generated any biologically active material.

Production of human and rat IFN-like protein in a mammalian expression system:

Several versions of the human and rat IFN-like protein have been produced in a mammalian expression system (either CHO or 293 cells). The proteins synthesized were either the native protein itself, or a native protein-Fc fusion. Some of the Fc fusion constructs contained a cleavage site which allows the native protein to be released from the Fc portion after being produced in the conditioned media of CHO cells.

PCR amplification of IFN-like molecule:
PCR primers were selected to amplify the coding sequence of rat/human IFN-like molecule:

Rat IFN-Like Molecule primers:

- IFN-Like molecule Fc-fusion:

1847-77 CCC AAG CTT ACC ATG ACA CTG AAG TAT TTA TG
Forward primer: Hind III site plus ATG
1847-78 AAG GAA AAA AGC GGC CGC ATT CAT GTT GAG TAG

- Reverse primer: Not I site and no stop codon for Fc fusion

Soluble IFN-like molecule:
1896-56 ACG CGT CGA CTC ATC AAT TCA TGT TGA GTA GTT TG
Reverse primer: Sal I site plus 2 stop codons (for pDSRα cloning).
1896-57 AAG GAA AAA AGC GGC CGC TCA TCA ATT CAT GTT GAG TAG
Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

Human IFN-like primers:

- Soluble human IFN-like primers:

1954-45 ACG CGT CGA CTT ATT ATT TCC TCC TGA ATA G
Reverse prime: Sal I site plus 2 stop codons (for pDSRα cloning).
1954-46 AAG GAA AAA AGC GGC CGC TTA TTA TTT CCT CCT GAA TAG AGC
Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

- Human IFN like-Fc fusion primers:

1955-44 CCC AAG CTT ACC ATG AGC ACC AAA CCT GAT ATG
Forward primer: Hind III site with 1st ATG
1954-47 CCC AAG CTT ACC ATG ATT CAA AAG TGT TTG TGG C
Forward primer: Hind III site with 2nd ATG
1954-48 AAG GAA AAA AGC GGC CGC ***GCG GCC CTC GAT*** TTT CCT CCT GAA TAG AGC TGT AA
Reverse primer: Not I site, no stop codon with ***Factor Xa*** cleavage site and Fc fusion
1954-49 AAG GAA AAA AGC GGC CGC TTT CCT CCT GAA TAG AGC TGT AA
Reverse primer: Not I site and no stop codon for Fc fusion

PCR Reaction:
Rat:
Reaction Mixture: template 20 ng, 1847-77 and 1847-88 or 1896-56/57, 20 pmol each, 1mmol dNTPs, 4mM MgCl2, 1X PCR buffer, 5u Taq polymerase.
Reaction condition: 2 cycle-linked PCR.
94 °C for 30 °C sec, 50 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94

°C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

Human interferon-like protein PCR conditions:
Reaction Mixture: template 20 ng, 1955-44 and 1954-45or 1954-46 (soluble form) or 1945-48/49 (Fc fusion), 20 pmol each, 1mmol dNTPs, 4mM MgCl2, 1X PCR buffer, 5u Taq plymerase.
Reaction condition: 2 cycle-linked PCR.
94 °C for 30 °C sec, 48 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94 °C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

While 1955-44 primer generates an ORF using first Met in the coding region, a separate PCR with 1954-47 to obtain an insert using 2nd downstream Met was also generated. But in terms of secretion efficiency, when tested in 293 EBNA transient transfection, there was no detectable difference could be defined.

For both rat and human, the PCR products were purified by Qiagen PCR purification spin column and subjected to restriction digestion by respective enzymes (HindIII and NotI (pCEP4) or SalI(pDSRα)). After digestion, the fragment was purified from agarose gel with Qiagen gel purification spin column. The purified fragment was quantified and ligated into pCEP4 (for native form), pCEP4-Fc (for Fc form) or pDSRα (native form or Fc form) vectors respectively. The ligation was transformed into DH10. The transformants were picked for miniprep and subsequent sequencing verification. Accuracy of each cloning fragment was verified by sequencing including the Fc junction sequence. The clone was then maxi-prepared for tissue culture transfection experiments. The IFN-Fc fragment in pCEP4-Fc vector can be released by cutting this vector with HindIII and SalI and re-ligated this fragment into pre-digested pDSRα to yield a vector suitable to transfect CHOD$^-$ cells.

Transfection:

- Protocol for transfeciton into 293 EBNA and CHO cells with lipofectin was adopted from the one used by Jin Cao. Same protocol was used to generate both transient and stable transfectants.
- A commercial available calcium phosphate transfection kit was used in CHO cell stable transfection (protocol is attached).
- A CHO cell transfection and selection protocol from Yi Luo was utilized, except calcium phosphate transfection procedure, which has a commercially available kit.
-

In general, lipofectin transfection yields more stable transfection colonies. Those colonies express comparable level of secreted proteins as those picked from calcium phosphate method.

Generate conditioned media containing recombinant protein.

In order to conduct functional studies on this interferon-like molecule, large quantity of conditioned media (CM) were generated from a pool of hygromycin selected 293 EBNA clones. The cells were cultured in Nunc Triple Flask (500cm) to 80% confluence before switching to serum free media for a week before harvesting. The CM was then sent to purification with protein A affinity chromatography. The purified protein was then used to generate a rabbit polyclonal antibody and to test for in vitro activities. The processing of signal peptide as well as partial amino acid sequence was verified by peptide sequencing.

Purification of human IFN-like-Fc

Conditioned media from CHO cells expressing hulFLM-Fc was thawed and 0.2μm filtered. The filtered material was loaded onto a Protein G column that was previously equilibrated with PBS, pH 7.0 . After loading, the column was washed with PBS until the absorbence at A_{280} reached baseline. The protein was eluted from the column with 0.1M Glycine-HCl pH 2.7 an dimmediately neutralized with 1M Tris-HCl pH 8.5. Fractions containing hulFLM-Fc were pooled and dialyzed into PBS and stored at -70°C.

Factor Xa cleavage of human IFN-like-Fc

The human IFN-like-Fc construct has a Factor Xa cleavage site (IEGR) inserted between the Fc and hulFLM. This site is cleaved with restriction protease factor Xa. The human IFN-like-Fc in PBS was dialyzed into 50mM Tris-HCl, 100mM NaCl, 2mM $CaCl_2$, pH 8.0. The Factor Xa was added to the dialyzed protein at 1/100 (w/w). The sample was incubated overnight at room temperature.

Abs (available, ordered, proposed):

1. Polyclonal:	Polyclonal antibodies were prepared using both rat and human proteins produced in E. coli and CHO cells (from above) using standard immunological techniques. Antisera were positive for the proteins as determined by Western blot analysis (standard techniques)
2. Monoclonal:	None.
3. Peptides:	None.

Phenotype and/or Biological Activity:

1. Transgenic /	(pending / analyzed)

other	
Because the lack of a phenotype constitutes a 'negative' result no conclusions can be drawn from this experiment. Further testing will be required to determine any or all of IFN-like proteins' biological activities in vivo.	
2. *in vivo* assays:	(available, used, proposed)
Not done.	
3. *in vitro* assays:	(available, used, proposed)
Rat IFN-like Fc fusion protein treatment of several cell lines caused phosphorylation of some cellular proteins (unidentified).	

References:

Nothing specifically published on this gene. Lots of references for the interferon family.

Genomic DNA Sequence (i.e. including all introns and exons):

The human gene was cloned from genomic DNA. The attached sequence (above) comes from genomic DNA and includes the coding region which is found in one exon, and the flanking regions.

Ortholog DNA Sequences:

Human and rat sequences cloned.